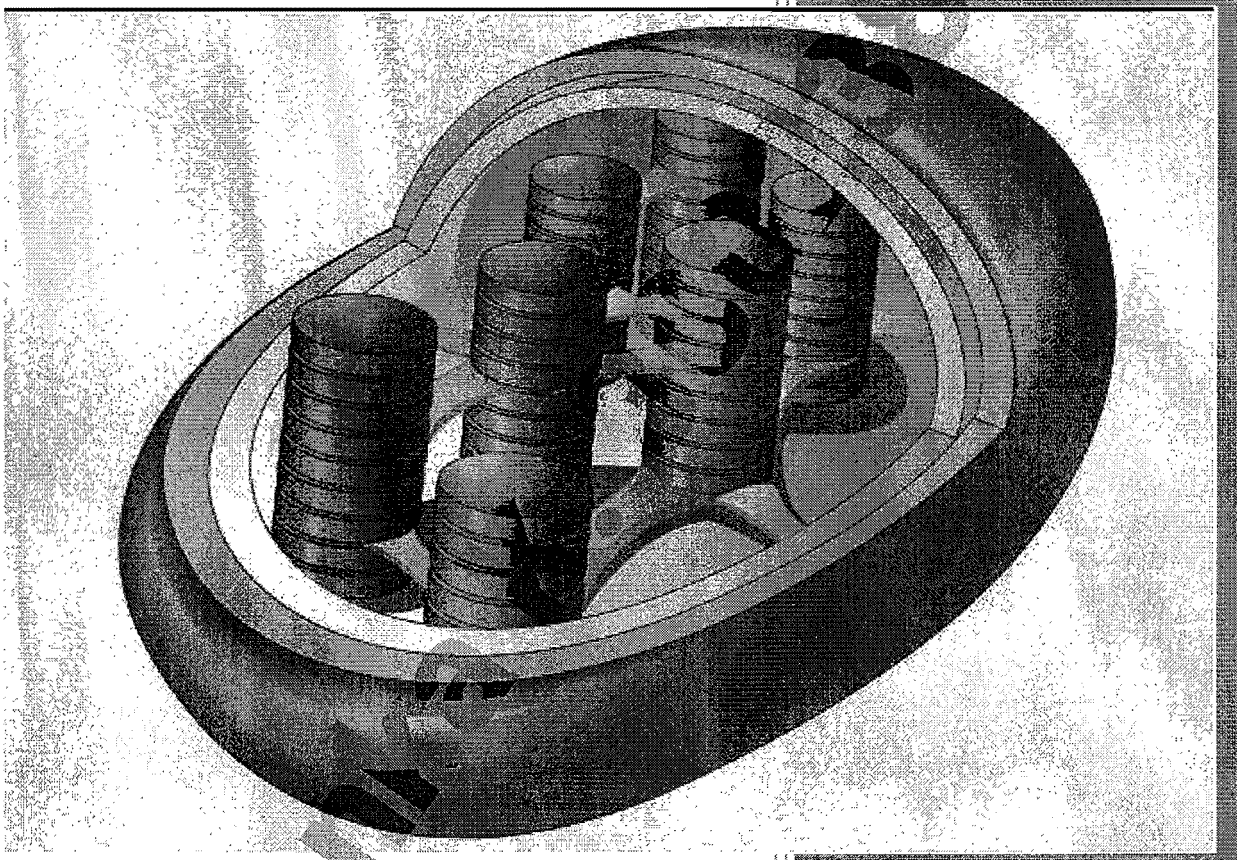
Evolution

2017

BOTANY: Plant Physiology



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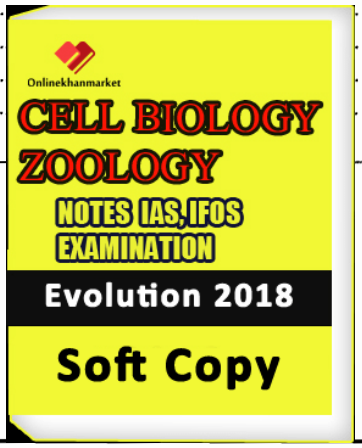


Water relations of plants

Importance of water in plant physiology & an introduction to plant water relation

Water is the most abundant constituent of all physiologically active plant cells. Leaves, for example, have water contents, which lie mostly within a range of 55–85% of their fresh weight. Other relatively succulent parts of plants contain approximately the same proportion of water, and even such largely nonliving tissues as wood may be 30–60% water on a fresh-weight basis. The smallest water contents in living parts of plants occur mostly in dormant structures, such as mature seeds and spores.

Water is essential in the plant environment for a number of reasons.

1. Water transports minerals through the soil to the roots where they are absorbed by the plant.
2. Water is also the principal medium for the chemical and biochemical processes that support plant metabolism. Water is a neutral and relatively non-reactive medium for biochemical reactions to proceed.
3. Under pressure within plant cells, water provides physical support for plants by means of turgidity. Turgidity is also the mechanism underlying the growth of plant cells and stomatal movement.
4. It also acts as a solvent for dissolved sugars and minerals transported throughout the plant.
5. In the process of photosynthesis, water serves the role of the ultimate donor of electrons, which get used for reducing carbon to generate carbohydrate in the Calvin Cycle.
6. In addition, evaporation within intercellular spaces provides the cooling mechanism that allows plants to maintain the favorable temperatures necessary for metabolic processes.

The great bulk of the water in any plant constitutes a unit system. This water is not in a static condition. Water is transported throughout plants almost continuously. There is a constant movement of water from the soil to the roots, from the roots into the various parts of the plant, then into the leaves where it is released into the atmosphere as water vapor through the stomatal transpiration. Thus, water is part of a ***hydrodynamic system***, which in terrestrial plants involves:

1. **Absorption** of water from the soil
2. Translocation throughout the plant (**Ascent of sap**)
3. Loss to the environment, principally in the process of **transpiration**. (Combined with evaporation from the soil and wet plant surfaces the total water loss to the atmosphere is called **evapotranspiration**.)

The above three processes constitute the major events of plant water relations. These events occur in a continuum that is known as the **Soil-Plant-Atmosphere Continuum (SPAC)**. It is the pathway for water moving from soil through the plant to the atmosphere. This continuum hypothesis characterizes the state of water in different components of the SPAC as expressions of the energy level or water potential of each (Fig. 1).

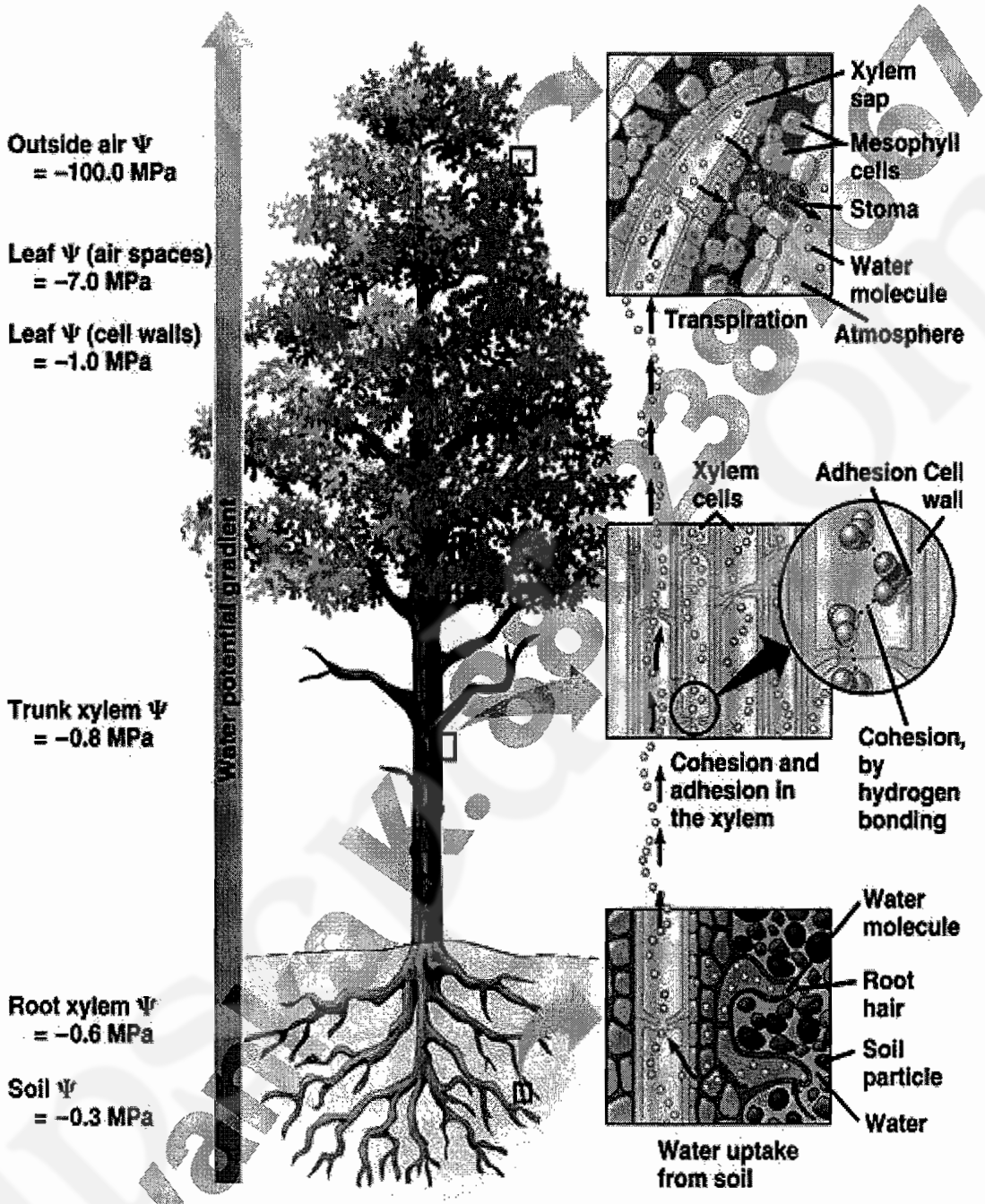


Figure 1: SPAC: The state of water in different components of the SPAC as expressions of the energy level or water potential

As illustrated in Fig. 1, water in the plant is in direct contact with water in the soil and with water vapour in the air around the plant. In this continuity of water that extends between the soil system and the atmosphere through a rooted plant, water moves from higher to lower water potentials.

Water enters a rooted land plant through the root. It travels across the root cortex to the root xylem, ascends in the xylem to the leaves, and is lost by evaporation from the surface of the mesophyll cells before diffusing

out through the stomata. The water potential of moderately dry air is much lower than that of the plant so there is a great tendency for water to leave the plant.

SPAC EVENT 1: Uptake of water by root

Water in the Soil

The water content and the rate of water movement in soils depend largely on soil type and soil structure. At one extreme, there is sand, in which the soil particles may be 1 mm or more in diameter. Sandy soils have a relatively low surface area per gram of soil and have large spaces or channels between particles. Hence, they have poor water retention. At the other extreme, there is clay, in which particles are smaller than 2 μm in diameter. Clay soils have much greater surface areas and smaller channels between particles. With the aid of organic substances such as humus (decomposing organic matter), clay particles may aggregate into "crumbs" that help improve soil aeration and infiltration of water.

The moisture-holding capacity of soils is called the **field capacity**. Field capacity is the water content of a soil after it has been saturated with water and excess water has been allowed to drain away. Clay soils or soils with high humus content have a large field capacity. A few days after being saturated, they might retain 40% water by volume. In contrast, sandy soils typically retain 3% water by volume after saturation.

The water potential of soils may be dissected into two components, the solute potential and the hydrostatic pressure. The solute potential (Ψs) of soil water is generally negligible because solute concentrations are low; a typical value might be –0.02 MPa. For soils that contain a substantial concentration of salts, however, Ψs is significant, perhaps –0.2 MPa or lower.

The second component of soil water potential is hydrostatic pressure (Ψp). For wet soils, Ψp is very close to zero. As a soil dries out, Ψp decreases and can become quite negative.

Water moves through soils predominantly by bulk flow driven by a pressure gradient. In addition, diffusion of water vapor accounts for some water movement. As plants absorb water from the soil, they deplete the soil of water near the surface of the roots. This depletion reduces Ψp in the water near the root surface and establishes a pressure gradient with respect to neighboring regions of soil that have higher Ψp values. Because the water-filled pore spaces in the soil are interconnected, water moves to the root surface by bulk flow through these channels down the pressure gradient.

The rate of water flow in soils depends on two factors:

1. The size of the pressure gradient through the soil, and
2. The hydraulic conductivity of the soil

Soil hydraulic conductivity is a measure of the ease with which water moves through the soil, and it varies with the type of soil and water content. Sandy soils, with their large spaces between particles, have a large hydraulic conductivity, whereas clay soils, with the minute spaces between their particles, have an appreciably smaller hydraulic conductivity.

Absorption of Water

Water is absorbed in rooted land plants mainly, but not exclusively, by the younger parts of roots in the regions of the root hairs. The root hairs are tubular extensions of epidermal cells and greatly increase the

available surface area for uptake of water and mineral salts. They form a very intimate relationship with soil particles and the inter-particulate capillary water. Although described variously in the past, plant physiologists call this region of the root ***the zone of rapid absorption***.

Water enters the root most readily in the apical part of the root that includes the root hair zone. More mature regions of the root often have an outer layer of protective tissue, called an exodermis or hypodermis, that contains hydrophobic materials in its walls and is relatively impermeable to water.

Intimate contact between the surface of the root and the soil is essential for effective water absorption by the root. This contact provides the surface area needed for water uptake and is maximized by the growth of the root and of root hairs into the soil. Root hairs are microscopic extensions of root epidermal cells that greatly increase the surface area of the root, thus providing greater capacity for absorption of ions and water from the soil. When 4-month-old rye (*Secale*) plants were examined, their root hairs were found to constitute more than 60% of the surface area of the roots.

As a root grows through the soil, new root hairs develop a short distance (usually 20-200mm) behind the meristematic zone and older root hairs die. This is actually a very efficient strategy to explore new areas of water resources by the plant.

As already discussed, in the soil, water is transported predominantly by bulk flow. However, when water comes in contact with the root surface, the nature of water transport becomes more complex. From the epidermis to the endodermis of the root, there are three pathways through which water can flow (Figure 2):

1. Apoplast
2. Symplast, and
3. Vacuolar pathways.

1. In the apoplast pathway, water moves exclusively through the cell wall without crossing any membranes. The apoplast is the continuous system of cell walls and intercellular air spaces in plant tissues. At the endodermis in the root, water movement through the apoplast pathway is obstructed by the Casparian strip. The Casparian strip is a band of radial cell walls in the endodermis that is impregnated with the wax-like, hydrophobic substance suberin. Suberin acts as a barrier to water and solute movement. Casparian strip breaks the continuity of the apoplast pathway, and forces water and solutes to cross the endodermis by passing through the plasma membrane. Thus, despite the importance of the apoplast pathway in the root cortex and the stele, water movement across the endodermis occurs through the symplast. In this way, the cells of the endodermis can control and regulate the movement of solutes through to the xylem. Such control is necessary as a protective measure against the entry of toxic substances, harmful disease-causing bacteria and fungi, and so on. As roots get older, the extent of suberin in the endodermis often increases. This blocks the normal exit of water and mineral salts from the cell. However, plasmodesmata may stay as pores in the cell walls, and 'passage cells' in which no extra thickening occurs also remain to allow water and solutes to pass through to the xylem.
2. The sympalstic or transmembrane pathway is the route followed by water that sequentially enters a cell on one side, exits the cell on the other side, enters the next in the series, and so on. In this pathway, water crosses at least two membranes for each cell in its path (the plasma membrane on entering and on exiting). Transport across the tonoplast may also be involved.

3. In the vacuolar pathway, water travels from one cell to the next via the plasmodesmata. The symplast consists of the entire network of cell cytoplasm interconnected by plasmodesmata.

Although the relative importance of the apoplast, trans-membrane, and symplast pathways has not yet been clearly established, experiments with the pressure probe technique indicate that the apoplast pathway is particularly important for water uptake by young corn roots (Frensch et al. 1996).

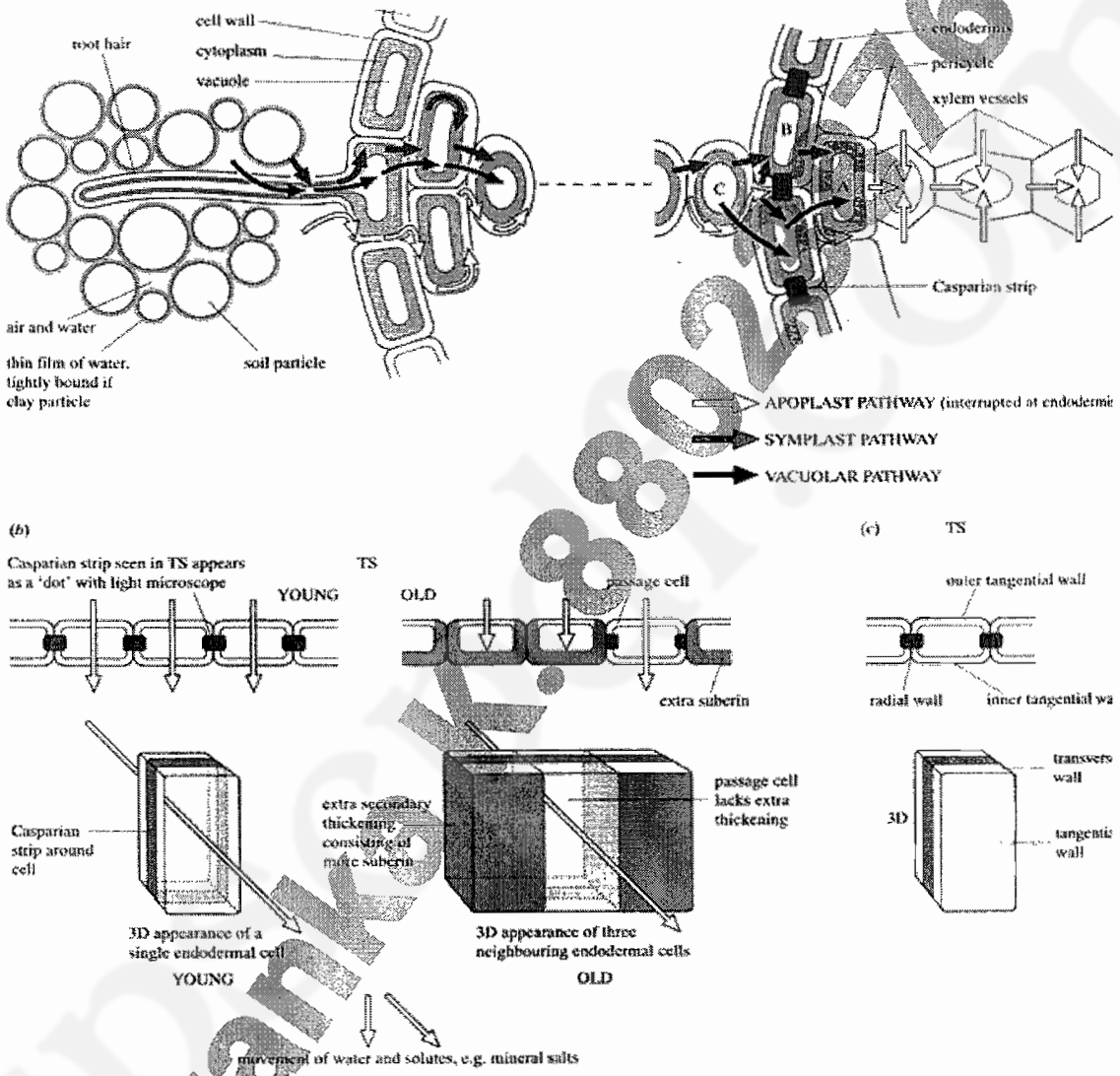


Figure 2: The apoplastic, symplastic and vacuolar pathways

The Driving Force for Absorption of Water

A water potential gradient exists across the root from higher potential in the epidermis to lower potential in the cells adjacent to the xylem. This gradient is maintained in three ways. However, the first mechanism is the primary one; the other two do not seem to contribute significantly in quantitative terms.

1. Water moving up the xylem in the transpirational pull sets up tension in the xylem and thus lowers the water potential of its sap. Whenever the water potential in the peripheral root cells is less than that of the soil water, movement of water from the soil into the root cells occurs. There is some

evidence that, under conditions of marked internal water stress arising due to transpiration, the tension generated in the xylem ducts will be propagated across the root to the peripheral cells. If this occurs, water potentials of greater negativity could develop in peripheral root cells than the water potential levels of the soil. This process is often called *passive absorption*, and it accounts for most of the absorption of water by terrestrial plants.

2. The phenomenon of *root pressure* represents another mechanism of the absorption of water. This mechanism is localized in the roots and is often called *active absorption*. Water absorption of this type only occurs when the rate of transpiration is low and the soil is relatively moist. Although the xylem sap is a relatively dilute solution, its solute concentration is still sufficient enough to allow a more negative water potential than what usually exists in the soil water. In other words, the xylem sap has a more negative (lower) solute potential than the dilute soil solution. This is because of deposition of mineral salts absorbed by the roots. A gradient of water potentials can thus be established, increasing in negativity across the epidermis, cortex, and other root tissues, along which the water can move laterally from the soil to the xylem.
3. In certain plants, there is tendency of dumping of osmotically active solutes through phloem translocation in the sub- epidermal cells, which brings down the levels of ψ_s in those cells.

Ultimately, all these conditions of ψ_w deficits are transferred to the epidermal cells with root hair projections and also within the apparent free space. This low ψ_w allows root hair to draw capillary water from the soil system.

Factors Affecting Water Absorption

Water uptake decreases when roots are subjected to low temperature, waterlogged soils or anaerobic conditions, or treated with respiratory inhibitors (such as cyanide). These treatments inhibit root respiration, and the roots transport less water. The exact explanation for this effect is not yet clear.

SPAC EVENT 2: ascent of sap

The Cohesion Tension Theory

The ascent of sap and its correlation to the transpirational pull is given by DIXON and JOLLY's proposal that is also known as COHESION TENSION THEORY [CTT]. It largely explains the ascent of sap in higher plants.

It relies on the physical properties of water, on mechanisms of liquid transport, and on the anatomical features of the xylem, the sap conducting system. The CTT actually embodies the work of several dozen scientists from around the world over the course of a century.

The CTT in its original proposal had two fundamental elements:

- **Cohesion**, among the molecules of water due to intermolecular Hydrogen Bonds, which provides strength to the water column to withstand negative pressure arising from transpirational pull.
- **Tension**, developed due to the transpirational pull, which is the driving force for ascent of xylary sap.

The CTT in its current form, as elaborated by several scientists across the world, can be summarized in the following eight statements:

1. Water within the whole plant forms a continuous network of liquid columns from the absorbing surfaces of roots to the evaporating surfaces mainly within the leaves.
2. Water columns within the conducting elements (vessels and tracheids) comprise about 99% of the whole water in a plant. The remaining 1% is constituted by the wall and cytoplasm of living cells.
3. The driving force for water movement in the system is generated by surface tension at the evaporating surfaces. The evaporating surface is at the wall of the leaf cells where evaporation takes place. This evaporation creates negative pressure, which is a suction force.
4. Because of surface tension evaporation lowers the water potential of the adjacent regions including the xylem elements. This change is instantaneously transmitted throughout the whole plant. Typical values can be as low as –3 MPa in crop plants, –4 MPa in trees, and –10 MPa in desert species.
5. Through the plant's vascular pathway, there is instantaneous transfer of the variations of tensions or pressure throughout the plant.
6. In this way, evaporation establishes gradients of pressure or tension along the pathway in transpiring plants. This causes an inflow of water from the soil to the transpiring surfaces.
7. Hydraulic continuity is highly dependent on the tensile strength of water.
8. Due to the fact that transpiration "pulls" the sap from the soil to the leaves, water in the xylem is in a metastable state of tension. In this state, the water column is susceptible to cavitation, (i.e., to the appearance of a vapor phase within the liquid phase). Whenever transpiration stops because of the absence of a humidity gradient between the leaf and atmosphere (as in the case when it rains period or in a night with high relative humidity), water will keep moving from the soil to the leaves until the difference of water potential across the water column disappears.
9. As long as transpiration occurs, the xylem sap is under tension and cavitation could take place. However, cohesion between water molecules, which gives water its tensile strength prevent cavitation, to a certain extent. During dry conditions in summer and frost-thaw cycles in winter, air bubbles can enter the xylem and reduce the hydraulic conductivity of the conducting elements.

Many experimental results provide strong support for the CTT. The CTT is a very well founded explanation of the sap ascent in plants, and has been verified by a large number of experiments.

Hydraulic Conductance Limitation Theory

This is a very relevant physiological – ecological synthesis put forward by Ryan & Yodder in 1997. Simply stated, this theory says that a tree would grow only as tall as its water conducting system allows it to.

Constraints on the capacity of the hydraulic system to deliver water to upper crown foliage might become increasingly limiting to stomatal conductance and photosynthesis as trees grow taller, thereby reducing carbon availability for further height growth. As trees grow taller, a larger soil-to-leaf water potential gradient is required to overcome the effect of gravity and the increased hydraulic resistance of a longer flow pathway. Low leaf water potential, however, causes a reduction in stomatal aperture, which reduces transpiration and counteracts further reduction in water potential, but also reduces photosynthesis. The postulated mechanism underlying the hydraulic conductance limitation hypothesis of Ryan and Yoder (1997) implies that trees should grow to a physiological "ceiling" height that reflects the tradeoff between turgor maintenance or cavitation avoidance on the one hand and photosynthetic carbon gain on the other, with the balance of these

factors being determined by the regulation of stomatal conductance. This theory is supported by experiments carried out on many tall trees.

SPAC EVENT 3: Transpiration

Transpiration is a process found in all the plants, except submerged aquatics, in which water is lost in the form of vapours from the living tissues of the aerial parts of the plant.

Although an evaporation process, transpiration is affected by other physical and physiological conditions prevailing in the plant. Whereas loss of water vapor can occur from any part of the plant which is exposed to the atmosphere, the great bulk of all transpiration occurs from the leaves.

Transpiration may occur from the following three sites.

1. Stomata: By evaporation of water from mesophyll cell surfaces and ultimate diffusion of the water vapour through stomata, the pores found in the epidermis of leaves and green stems. In tropical regions, about 90% of the water is lost this way.
2. Cuticle: By evaporation of water from the outer walls of epidermal cells through the waxy cuticle covering the epidermis of leaves and stems. About 10% of the water lost, varying with thickness of cuticle. In ferns, where the thickness of the cuticle is relatively less, cuticular transpiration can account for ~40% of the total water loss. Cuticular transpiration becomes important in the flowers and fruits.
3. Lenticels: By evaporation of water through lenticels. These are small slits in the stems and bark of trees for gas exchange. It accounts for minute proportions, although this becomes the main method of water loss from deciduous trees after leaf fall.

Why does transpiration occur?

Transpiration is a necessary consequence of the relation of water to the anatomy of the plant, and especially to the anatomy of the leaves. Terrestrial green plants are dependent upon atmospheric carbon dioxide for their survival. In terrestrial vascular plants, the principal carbon dioxide–absorbing surfaces are the moist mesophyll cells walls which bound the intercellular spaces in leaves. Ingress of carbon dioxide into these spaces occurs mostly by diffusion through open stomates. When the stomates are open, outward diffusion of water vapor unavoidably occurs, and such stomatal transpiration accounts for most of the water vapor loss from plants. Transpiration is, thus, an incidental phenomenon.

Water normally leaves the plant as water vapour. The change from a liquid state to a vapour state requires the addition of energy, which is provided by the Sun, and this energy only maintains the transpiration stream. The quantities of water lost by transpiration can be very large. A herbaceous plant, such as cotton or sunflower, can lose between 1-2 dm^3 of water per day, and a large oak tree may lose more than 600 dm^3 per day.

The phenomenon of transpiration is best studied in angiosperm crop plants where there is an immense economic aspect involved to it. It is well established through a number of experimental studies that if the water lost by transpiration is not adequately replenished by soil water or irrigation, the resulting situation may cause irreversible physiological damage to the plant. The losses can be great if a standing crop of an economically important plant suffers such damage. In the history of humanity, many devastating famines have occurred because the rainfall in a given year was not adequate to replenish the water lost by

transpiration. In modern world, such situation has been sought to be averted by irrigation and physiological interventions.

Transpiration and Movement of Water through the Leaf

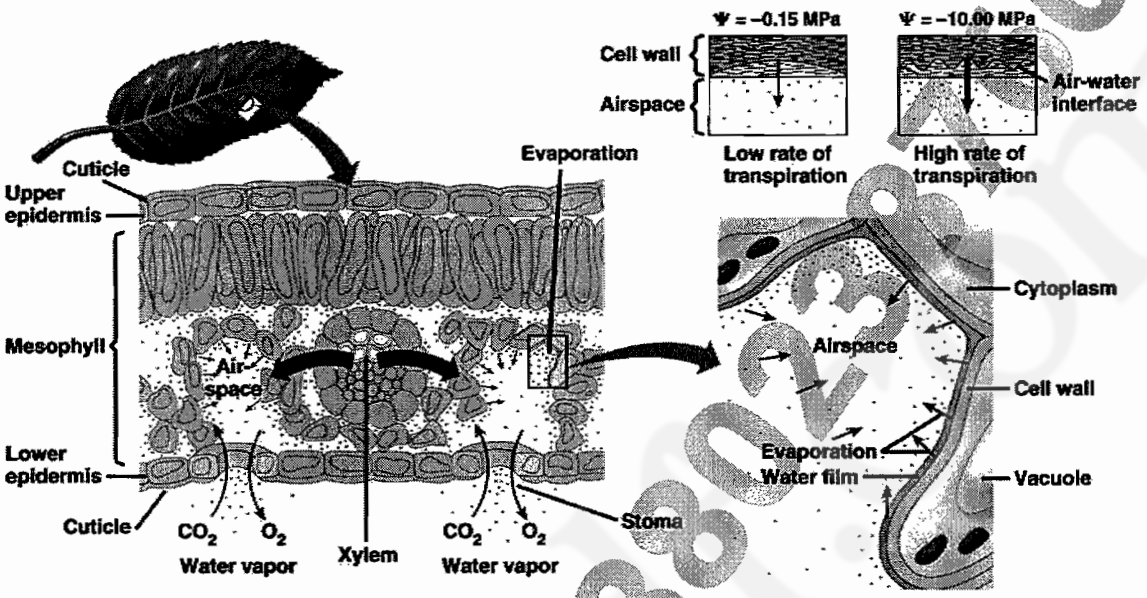


Figure 3: Movement of water through the leaf

Water is brought to the leaf in the xylem vessels due to the transpirational pull. The xylem is part of the vascular bundles, which spread to form a fine branching network throughout the leaf. The branches end in one or a few xylem vessels that possess almost no lignification. Water can therefore escape easily through their cellulose walls to the mesophyll cells of the leaf.

There can be three pathways which water can then follow, namely the apoplast pathway (cell walls), the symplast pathway (cytoplasm and plasmodesmata) and the vacuolar pathway (from vacuole to vacuole).

The apoplast pathway: The apoplast is the system of adjacent cell walls, which is continuous throughout the plant. Up to 50% of cellulose cell wall may be 'free space' that can be occupied by water. As water evaporates from the mesophyll cell walls into the intercellular air spaces, tension develops in the continuous stream of water in the apoplast, and water is drawn through the walls in bulk flow by the cohesion of water molecules Water in the apoplast is supplied from the xylem.

The symplast pathway: The symplast is the system of interconnected protoplasts in the plant. The cytoplasm of neighbouring protoplasts is linked by the plasmodesmata. Once water, and any solutes it contains, is taken into the cytoplasm of one cell, it can move through the symplast without having to cross further membranes. Movement might be aided by cytoplasmic streaming. The symplast is a more important pathway of water movement than the vacuolar pathway.

The vacuolar pathway: In the vacuolar pathway, water moves from vacuole to vacuole through neighbouring cells, crossing the symplast and apoplast in the process and moving through membranes and tonoplasts by osmosis.

The water potential gradient within a leaf

Water evaporates from the wet walls of the mesophyll cells into the intercellular air spaces, particularly into the larger **substomatal air spaces**. Loss of water from a cell would result in a decrease in its pressure potential and its water potential. An adjacent cell would then have a higher water potential than this cell. Water will therefore move from one cell to its neighbouring cell, thus lowering the water potential of other neighbouring cells as well. In this way, a gradient of water potential is set up across the leaf resulting in a higher potential in the xylem and a lower potential in the mesophyll cells. Water continuously enters the mesophyll cells from the xylem by osmosis.

It has sometimes been suggested that water moves across the leaf in response to a gradient of solute potentials. However, although a water potential gradient exists, there is no evidence to suggest that solute potentials of the cells differ significantly from one another. Differences in water potential are due mainly to differences in pressure potential, since loss of a small amount of water from a cell has a much greater effect on pressure potential than on solute potential. The same applies to the root where gradients of pressure potential and water potential exist, but not necessarily of solute potential. Even if a solute potential plays a role in the roots, as suggested by many recent evidences – it is only of secondary importance.

Exit of Water through Stomata & the Diffusion Shell

The three pathways described above ultimately end with water evaporating from the cell surfaces and intercellular spaces into air spaces. From here, **water vapour diffuses out through the stomata, from a high water potential inside the leaf to a much lower one outside the** leaf. In dicotyledons, stomata are usually confined to, or are more numerous in, the lower epidermis.

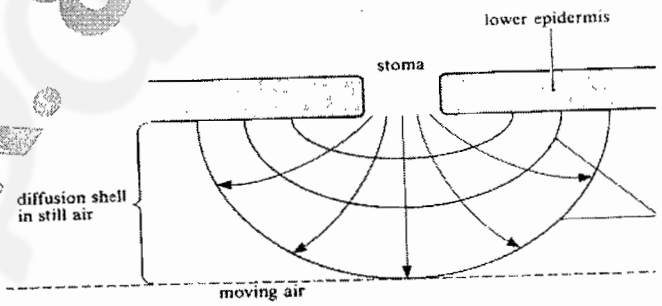


Figure 4: Diffusion Shell

of water molecules

Immediately next to the leaf is a layer of stationary air whose thickness depends on the dimensions and surface features of the leaf, such as hairiness, and also on wind speed. Water vapour must diffuse through this layer before being swept away by moving air (mass flow). The thinner the stationary layer (which is usually caused by relative humidity, wind velocity & temperature) the faster is the rate of transpiration. So theoretically, each stoma has a diffusion gradient, or '**diffusion shell**' around it (Fig. 4). In practice, the diffusion shells of neighbouring stomata overlap in still air to form one overall diffusion shell.

Effects of Environmental Factors on Transpiration

Plants show many features, which enable them to reduce loss of water by transpiration in dry conditions. Such features are described as xeromorphic. Plants growing in dry habitats and subjected to drought are called xerophytes and possess many xeromorphic features. Plants growing under conditions in which there is normally an adequate water supply ate called mesophytes, but can show some xeromorphic features.

Temperature

The external factor that has the greatest effect on transpiration is temperature. The higher the temperature, the greater the rate of evaporation of water from mesophyll cells and the greater the saturation of the leaf interior with water vapour. At the same time, a rise in temperature lowers the relative humidity of the air outside the leaf. Both events result in a steeper water potential gradient from leaf interior to external atmosphere and lead to a faster rate of diffusion. Alternatively, it can be said that water potential increases inside the leaf while decreasing outside the leaf because of high temperature.

The temperature of the leaf is raised by solar radiation. Pale-coloured leaves reflect more of this radiation than normal leaves and therefore do not heat up as rapidly. The pale colour is usually due to a thick coat of epidermal hairs, waxy deposits, or scales, and is a xeromorphic feature.

Humidity and vapour pressure.

Low humidity outside the leaf favours transpiration because it makes the diffusion gradient of water vapour (or water potential gradient) from the moist leaf interior to the external atmosphere steeper. As the concentration of water vapour in the external atmosphere, that is the humidity, rises, the diffusion gradient becomes less steep. Water potential of the atmosphere also decreases with altitude as atmospheric pressure decreases. High altitude plants therefore often show xeromorphic adaptations to reduce transpiration rates. A xeromorphic feature of some leaves is the presence of sunken stomata, that is stomata in grooves or infoldings of the epidermis, around which a high humidity can build up and reduce transpiration losses.

Air movement

In still air a layer of highly saturated air builds up around the leaf, reducing the steepness of the diffusion gradient between the atmosphere inside the leaf and the external atmosphere. Any air movement will tend to sweep away this layer. Thus windy conditions result in increased transpiration rates, the increase being most pronounced at low wind speeds. High winds may result in closing of the stomata, stopping transpiration.

Hairs and scales trap still air, tending to reduce transpiration rates.

Light

Light affects transpiration because stomata usually open in the light and close in darkness. Blue Light is now known to be a signaling factor for stomatal opening. [Refer to your notes on Stomatal Physiology]. At night, therefore, only small amounts of water are lost (through the cuticle or lenticels). As stomata open in the morning, transpiration rates increase.

Effect of Plant or Internal Factors on the Rate of Transpiration

The effects of some xeromorphic adaptations on transpiration rates have been considered above. Further examples of the ways in which such 'internal' as contrasted to 'external' (environmental) factors can operate are given below.

Leaf surface area and surface area to volume ratio

Transpiration of a plant increases with its total leaf surface area, and with leaf surface area to volume ratio. Reduction of leaf surface is achieved when leaves are reduced to needles, such as in Pinus and other conifers, or to spines, as in cacti. The shedding of leaves in dry or cold seasons by deciduous plants is a xeromorphic adaptation. In cold seasons, water may be unavailable through being frozen in the soil.

Surface area to volume ratio can be reduced by using the stem as the main photosynthetic organ, as in cacti.

Cuticle

The cuticle is a layer that is secreted by the epidermis. It consists of a fatty substance called cutin, which is relatively waterproof. In general, the thicker the cuticle the lower the rate of transpiration through it. Where it is thin, as in ferns, 30-45% of the transpiration losses can be through it.

The upper surfaces of dicotyledonous leaves, which are exposed to direct sunlight and are less protected from air currents than the lower surfaces, often possess thicker cuticles than the lower surfaces. Increased wax deposits on leaves can virtually eliminate cuticular transpiration. In addition, waxy leaves are usually shiny and so reflect more solar radiation.

Stomata

In general, the greater the number of stomata per unit area, the greater is the rate of stomatal transpiration; however, their distribution is also important. For example, the lower surfaces of dicotyledonous leaves usually have more stomata than their upper surfaces, whereas monocotyledonous leaves as in maize and oat, which are generally held vertically rather than horizontally, have similar upper and lower surfaces with similar stomatal distributions.. On average, fewer stomata occur in plants adapted to dry conditions. The number may vary within the same species as a result.

Age of plants

Rate of transpiration is slow at the seedling stage, maximum at maturity and gradually decreases near the senescence. These differential rates of transpiration are clearly established in most of the angiosperm plants studied.

Functions of Transpiration

Transpiration has been described as a 'necessary evil' because it is **inevitable, but potentially harmful**. It happens because of the existence of wet cell walls from which evaporation occurs. **Water vapour escapes mainly through the open stomata that are essential for gaseous exchange between the plant and its environment.** We cannot expect a higher land plant to survive if it does not take up CO_2 from the atmosphere through its stomata. The carbon dioxide thus obtained is essential for photosynthesis. Since there is much more oxygen than carbon dioxide in the atmosphere, plants can get all the oxygen they need even with stomata shut. They therefore respire in the dark just as efficiently as they do in the light. If there was no cuticle, stomata would be unnecessary and gaseous exchange would be even more efficient. However, loss of water could not then be controlled. The cuticle reduces water loss and further control is exercised by the stomata, which in most plants are highly sensitive to water stress and close under conditions of drought. They also usually close during the night when photosynthesis ceases. Loss of water can lead to wilting, serious desiccation, and often death of a plant. There are evidences that even mild water stress results in reduced growth rate and causes economic losses in crops through reductions in yield.

Although it is inevitable, it is worth asking whether there might be some advantages associated with transpiration. Some possibilities are as follows.

1. The evaporation of water from mesophyll cells that accompanies transpiration requires energy and therefore results in cooling of the leaves in the same way that sweating cools the skin of mammals. This is sometimes important in direct sunlight when leaves absorb large amounts of energy and

experience rises in temperature, which, under extreme conditions, can inhibit photosynthesis because of denaturation of enzymes. However, it is unlikely that the cooling effect is of significance under normal conditions. Plants that live in hot climates usually have other means of avoiding heat stress.

2. It has been suggested that the transpiration stream is necessary to distribute mineral salts throughout the plant, since these move with the water. While this is true, the reality is that very low transpiration rates would be sufficient. For example, mineral salt supply to leaves is just as sufficient at night, when transpiration is low, as during the day because the xylem sap is more concentrated at night. Uptake of mineral salts from the soil is largely independent of the transpiration stream.
3. Transpirational stream serves the physiologically active leaf cells with the water they need for various biochemical reactions, especially photosynthesis. **Winneberger** suggested that the water supply to various plant organs by the transpirational pull is an essential process to ensure normal vegetative & reproductive growth.

Diversity of Adaptive Features to Minimize Transpiration

Following three types of features are found in plants to reduce transpiration:

1. Morphological features.
2. Anatomical features.
3. Physiological features.

Morphological Features

1. Presence of dry, hard and cylindrical stem *e.g., Salvadora* and *Leptadaenia.* Some stems possess ridges and furrows *e.g., Casuarina.*
2. Presence of bark and cork on stems *e.g., Acacia.* .
3. Reduction in number of branches and size of stems. Some stems become flat and fleshy *e.g., Opuntia.*
4. Decrease in length and number of branches in roots. They reach deeply in the soil. *e.g.,Alfalfa.*
5. Presence of fleshy roots for storage of water *e.g., Asparagus.*
6. Presence of small scaly and reduced leaves e.g., *Ruscus* and *Tamanx.*
7. Presence of quite small leaves which soon falloff *e.g., Capparis* and *Euphorbia.*
8. Division of leaves into many small leaflets or lamina *e.g., Acacia.*
9. Presence of long narrow and needle like leaves *e.g., Pinus.*
10. Presence of thick leaves covered by thick waxy layer or cuticle. *e.g., Calotropis, Ficus* and *Salvadora.*
11. The leaves of some plants become thick, fleshy and mucilagenous or possess resin or gum *e.g., Aloe & Agave.*
12. Modification of stipules into thorn *e.g., Ziziphus* and *Acacia.*
13. Presence of excess hairs or hairy covering on the leaves, flowers and stems *e.g.,Gnephalium, Aerva, Calotropis.*
14. Rolling and folding of leaves *e.g., Marrum grass.*

15. Presence of smooth and shining leaves *e.g., Nerium.*
16. Production of gum, resin and mucilage etc. in the cells of plants *e.g., Pinus, Opuntia* and *Cycas.*

Anatomical Features

1. Presence of thick cuticle *e.g., Agave, Dianthus, Ficus* and *Nerium* etc.
2. Presence of another waxy layer on the cuticle *e.g., Salix glaucophylla*
3. Presence of multilayered epidermis *e.g., Nerium, Ficus* and *Pepromia.*
4. Presence of hairs and scales on the epidermis.
5. Presence of sunken and less number of stomata. *e.g.,* most of the xerophytes and sometimes in the depressions of epidermis *e.g., Nerium.*
6. Presence of mucilage, gum, resin, latex and tanin in the cells of hypodermis and cortex *e.g., Opuntia, Pinus, Ficus, Euphorbia* and *cycas.*
7. Presence of exess amount of sclerenchyma *e.g., Salvadora*
8. Presence of compactly arranged palisade cells *e.g., Cycas* and *Nerium.*
9. Formation of cork and bark *e.g., Acacia, Ziziphus* and *Prosopis* etc.
10. Formation of cork layer in the stem.

Physiological Features

1. Presence of high OP in the cell-sap of leaves.
2. Presence of hydrophylic substances in the cells of stems and leaves which retain water and become fleshy.
3. Presence of excessive growth in the roots due to which they become quite long and reach at a greater depth to absorb the water *e.g., Alhagi camelorun.* .
4. Closing of stomata during adverse conditions of environment.
5. Forced closure of stomata by abscissic acid under dry conditions

Anti-transpirants

It is obvious that almost all the water absorbed by the plants is practically lost by the process of transpiration. To avoid the excessive water loss and the accompanying physiological damage substances that can reduce the transpiration without hindering the other processes have become crucially important. These substances are termed as antitranspirants. An antitranspirant is, therefore, a substance applied to the plant for reducing transpiration without causing significant effect on other plant processes, such as photosynthesis and growth.

Several substances *viz.*, colourless plastics, silicone oils, low viscosity waxes have been used but failed to give promising results. These substances make a colourless transparent film over the surface of leaf that is permeable to O_2 and CO_2 but not to water vapours. This is the physical approach to transpiration control.

The fungicide phenylmercuric acetate when sprayed at 10^{-4} M concentration resulted into a partial closure of stomata for about 2 weeks, but this substance has certain toxic effects on fruits and vegetables. Similarly, 'aspirin' and abscisic acid (ABA) also induce closure of stomata when applied to the leaves. These strategies

are grouped under physiological approach to transpirational control. These substances have comparatively little toxic effects, but they are costly and cannot be frequently used. Carbon dioxide (CO_2) is yet another effective antitranspirant when used at a concentration slightly higher than the natural O.03% in the atmosphere. It cannot be effectively used because higher concentration of CO_2 causes complete closure of stomata and affect the process of photosynthesis. However, plant physiologists are trying to evolve a perfect antitranspirant, which should solve the problem.

The water potential concept

Water potential is the potential energy of water relative to pure water in reference conditions. It quantifies the tendency of water to move from one area to another due to osmosis, gravity, mechanical pressure, or capillary action. Water potential is measured in units of pressure and is commonly represented by the Greek letter Ψ (Psi). This concept has proved especially useful in understanding water transport within plants, animals, and soil.

Typically, pure water at standard temperature and pressure (or other suitable reference condition) is defined as having a water potential of 0. The addition of solutes to water lowers its potential (makes it more negative), just as the increase in pressure increases its potential (makes it more positive). If possible, water will move from an area of higher water potential to an area which has a lower water potential.

One very common example is water that contains a dissolved salt, like sea water or the solution within living cells. These solutions typically have negative water potentials, relative to the pure water reference. If there is no restriction on flow, water molecules will proceed from the pool of pure water to the more negative water potential of the solution. This effect can be used to power a osmotic power plant.

Simple Systems

Many different potentials affect the total water potential, and these effects are additive. In a simple system, two components are the pressure potential (Ψ_p) and the solute potential (Ψ_π sometimes also Ψ_s). In this simple system, the water potential is given by the following formula:

$$\Psi = \Psi_p + \Psi_\pi$$

Pressure potential

Pressure potential is based on mechanical pressure, and is an important component of the total water potential within plant cells. Pressure potential is increased as water enters a cell. As water passes through the cell wall and cell membrane, it increases the total amount of water present inside the cell, which exerts an outward pressure that is retained by the structural rigidity of the cell wall.

The pressure potential in a living plant cell is usually positive. In plasmolysed cells, pressure potential is almost zero. Negative pressure potentials occur when water is pulled through an open system such as a plant xylem vessel. Withstanding negative pressure potentials (frequently called *tension*) is an important adaptation of xylem vessels.

Solute Potential

Pure water is usually defined as having a solute potential (Ψ_π) of zero, and in this case, solute potential can never be positive. The relationship of solute concentration (in molarity) to solute potential is given by the van 't Hoff equation:

$\Psi_\pi = - miRT$

where m is the concentration in molarity of the solute, i is the van 't Hoff factor, the ionization constant of the solute (1 for glucose, 2 for NaCl, etc.) R is the ideal gas constant, and T is the absolute temperature.

For example, when a solute is dissolved in water, water molecules are less likely to diffuse away via osmosis than when there is no solute. A solution will have a lower and hence more negative water potential than that of pure water. Furthermore, the more solute molecules present, the more negative the solute potential is.

Solute potential has important implication for many living organisms. If a living cell with a lower solute concentration is surrounded by a concentrated solution, the cell will tend to lose water to the more negative water potential of the surrounding environment. This is often the case for marine organisms living in sea water and halophytic plants growing in saline environments. In the case of a plant cell, the flow of water out of the cell may eventually cause the plasma membrane to pull away from the cell wall, leading to plasmolysis.

Complex Systems

There are other contributors to water potential, and their contribution is given by the following equation:

$\Psi = \Psi_0 + \Psi_\pi + \Psi_p + \Psi_g + \Psi_v + \Psi_m$

where Ψ_0 is the reference correction, Ψ_π is the solute potential (discussed above), Ψ_p is the pressure potential (discussed above) Ψ_g is the gravimetric component, Ψ_v is the potential due to humidity, and Ψ_m is the potential due to matrix effects (eg, fluid cohesion and surface tension.)

Stomatal Movement

Studies on stomatal physiology

- Late 17 th century: First reported to be present on the green, aerial parts of the plant by M. Malpighi
- 1856: Hugo von Mohl suggested that turgor changes in the guard cells cause stomatal movement
- 1908: FE Lloyd gave Starch Sugar interconversion model
- 1943: S. Imamura gave K+ ion fluxes model, which is the largely acceptable model today.
- 1973: Krikorian *et al* for the first time reported Radial Micellation (RM) in the Guard Cell wall. RM is the ultrastructural basis of differential swelling of the Guard Cells under turgid conditions, leading to stomatal opening.
- 1980s, 90s and recent years – There are 2 major groups contributing to our current knowledge on stomata:
  - Shimazaki *et al*
  - E. Zaiger & Talbott

  They have shown that
  1. Stomatal opening is primarily controlled by ionic fluxes as originally suggested by Imamura
  2. The events leading to ionic fluxes are initiated by blue light signaling. The blue light receptor is Zeaxanthin
  3. Red light also plays a supporting role, by photosynthetic mediation and also photosynthesis independent.

Distribution of the stomata

In the plant world

- All the Pteridophytes, Gymnosperms and Angiosperms on most of the pigmented aerial structures; leaves, floral leaves, green stem epidermis etc. Submerged aquatics are exception.
- Among Bryophytes, only the sporogonium bears functional stomata and that too in two groups – Mosses and Hornworts

On an angiosperm leaf

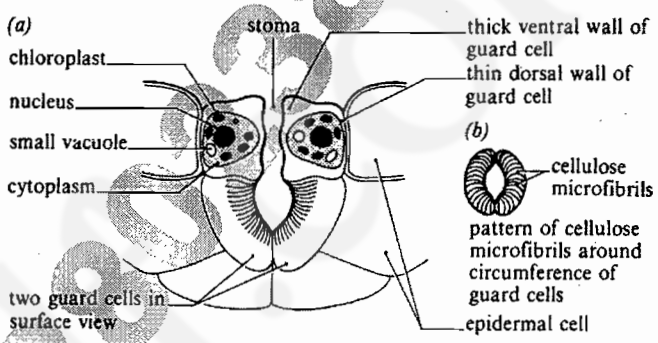
The major patterns are:

1. Submerged aquatics: No stomata on the leaves
2. Aquatics with exposed leaves: Stomata on the exposed surface; e.g. on the upper surface of the leaf in *Lilium*
3. Terrestrial monocots: Stomata on both the sides, almost equal density (50 – 200 /mm^2), e.g. *Allium, Hordeum, Triticum,* Maize

4. Herbaceous dicots: Stomata on both the sides, but greater density on the lower surface of the leaves (30 – 120 / mm² on the upper surface, while 150 – 250 / mm² on the lower surface). e.g. *Nicotiana, Medicago, Pelargonium*
5. Woody species: Stomata almost exclusively on the lower surface of the leaf (200 – 800 / mm²) seen in *Quercus, Tilia*

Functional anatomy of guard cells

A variety of external and internal stimuli affect the opening and closing of stomata by altering the size of stomatal pores. The important among them are light and dark, CO_2 concentration, water supply, *p*H of the cell sap, etc. In most of the cases when water supply is adequate, the stomata tend to open during daytime in response to light and close at night. The opening and closing of stomata operates because of turgor changes in the guard cells. When the guard cells become turgid, their thin walls get extended and thick walls become slightly concave so that the stomatal aperture opens. On the other hand, the guard cells become flaccid when they lose water. Their thick walls revert to original position resulting in the closure of stomatal pore.



Investigations on submicroscopic anatomy of guard cell walls suggest that the special orientation of cellulose micro fibrils and micelles are mainly responsible for opening and closing of stomatal pores (Krikorian et. al., 1973). These studies have shown that cellulose micro fibrils and micelles are arranged around the circumference of the elongated guard cells. A GREATER DENSITY OF RADIATING MICRO FIBRILS ON THE VENTRAL WALLS MAKES THEM LESS FLEXIBLE. This arrangement is called **radial micellation**. Such guard cells, when take up water and expand, cannot increase much in diameter because the micro fibrils on the ventral walls do not stretch much. On the other hand, they increase in length. Since the two guard cells remain attached to each other at both ends, they bend outward on swelling and result opening of stomatal pore.

Stomatal physiology

H. von Mohl in 1856 suggested that turgor changes in the guard cells provide the basis of stomatal opening. This postulation was consistent with the anatomical uniqueness of the guard cell walls. However, plant physiologists working on the stomatal function during early 20th century were intrigued about osmoregulation in the guard cells. A number of theories were proposed to explain the osmoregulation in the guard cells, out of which **Starch - Sugar Interconversion Hypothesis** proposed by FE Lloyd in 1908 remained acceptable for a very long period. **S Imamura [1943]** for the first time reported K^+ fluxes in the guard cells, which regulate their osmotic behaviour. Following that, the recent works of **Shimazaki [1986, 1997], Zeiger & Talbott [1998, 2003]**, and **MB Wilkins [2002]** give us a much better and detailed understanding of the process. It is worth emphasizing that the starch – sugar interconversion hypothesis is not a valid explanation for the process in its entirety, but this definitely partially accounts for the stomatal opening.

The currently accepted view

Although stomata open for the sole purpose of CO_2 uptake by the plants, CO_2 concentration itself does not regulate the stomatal opening.

Through a number of field observations and experimental studies, it is well established now that stomatal opening is a light mediated process where two complementary pathways take place. One of them is photosynthetic and driven by red light. The second pathway is non-photosynthetic mediated by blue light. The red light opens the stomatal apertures by a process that is essentially linked to photosynthesis in the guard cells.

- Experimental studies show that stomatal openings result when a leaf surface is supplied with a red beam of light. Moreover, the stomatal opening by red light may be inhibited by the application of photosynthetic inhibitors [such as Dichlorophenyldimethylurea, DCMU]. However, such inhibition is never absolute, which suggests that there exists some other non-photosynthetic mechanism to regulate the opening of stomata.
- Moreover, in a number of plant species key enzymes of the Calvin cycle are missing in the guard cells. It supports the view that the photosynthesis-mediated pathway is only a secondary strategy to regulate the stomatal movement.
- There are many direct evidences too suggesting that there exist two separate light driven pathways regulating the stomatal movements.
- In one of the experiments, red light was first applied to the leaf surface continuously for some time. Stomatal opening occurred but the effect of the red light showed a saturation point in the sense that the stomata would not open further even if an additional amount of red light was supplied. The saturation effect could be broken by application of blue light, which caused rapid further opening of stomata. Since blue light is not favourable to drive photosynthesis, it suggests that some non-photosynthetic pathway mediated by blue light also exists.
- *Now it is established that blue light is the dominant signal that sets on an osmotic process leading to the eventual opening of the stomatal apertures. Blue light affects the stomatal physiology in a rapid and reversible way by acting only on the guard cells. The guard cells have a carotenoid photoreceptor pigment, ZEAXANTHIN, for the blue light perception. This photoreceptor gives rise to a signal transduction cascade that links the incidence of blue light on the leaf surface to stomatal opening.*
- The basic outline of the process is something like this:

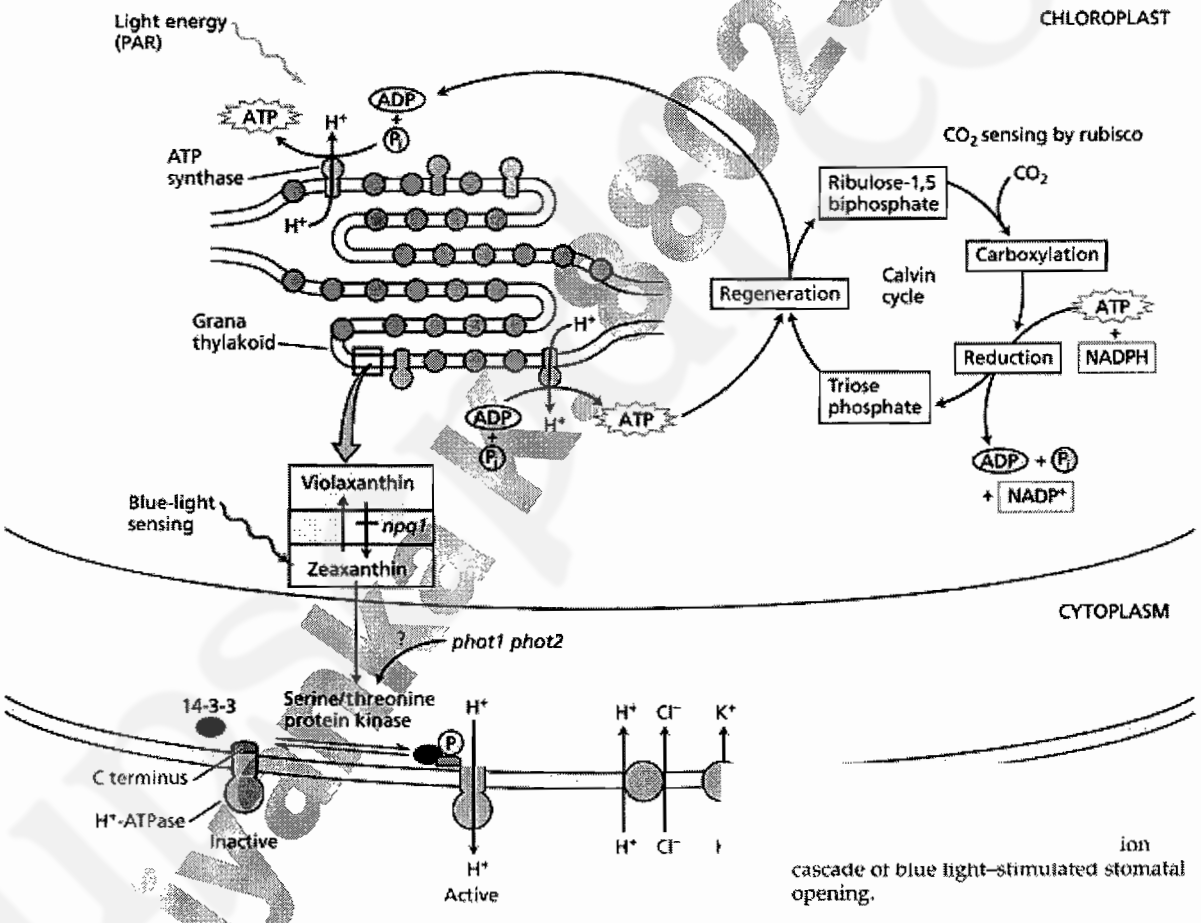
Blue light received by the Zeaxanthin → Uptake of ions by guards cells + accumulation of organic solutes in the guard cells → Establishment of **low** ψ_w in the guard cell → Osmotic uptake of water by the guard cells from the neighbouring cells → Increased turgidity → Curved stretching of the guard cells because of unevenly thickened walls → Opening of the stomata.

How blue light induces accumulation of ions and organic solutes in the guard cells

Experiments on isolated guard cell protoplasts reveal that:

1. Once the Zeaxanthin molecule perceives blue light it initializes a signal cascade within the guard cell. The best results are obtained when blue light is provided with a background of red light.
2. The signal cascade is completed in 15 - 30 seconds and it eventually activates a proton pumping ATPase complex in the plasma membrane.
3. The signal cascade is not yet fully understood but the currently accepted model is as follows:

Excitation of Zeaxanthin by blue light → Binding of activated Zeaxanthin with kinases (mainly encoded by *Phototropin* or ***phot*** genes) present near the plasma membrane → Activation of the kinases → Phosphorylation of the C-terminus inhibitory sub-unit of the plasma membrane ATPase/ H^+ pump → Phosphorylated C-terminus inhibitory sub-unit is sequestered with help of 14-3-3 proteins → Removal of the C-terminus inhibitory sub-unit activates the ATPase/H^+ pump → Breakdown of ATP → Pumping out of H^+ ions by the activated ATPase / H^+ pump complex.



ion cascade of blue light–stimulated stomatal opening.

4. A vigorous pumping out of H^+ starts from the guard cell, creating a charge imbalance inside the cell, which will be more negative now. This pumping out of H^+ consumes ATP. The ATP consumed here comes from photo-phosphorylation that is promoted by red light. This explains the maximal stomatal opening by a combined effect of blue and red light.

5. There is one more role for the red light. During the red light driven photosynthesis, Zeaxanthin also accumulates within the guard cell. Zeaxanthin attaches to kinases to produce a functional photo-signaling unit. Without a background red light, the guard cells have been shown to have decreased sensitivity towards blue light. This is probably because there are not enough Zeaxanthin molecules to generate the signal cascade. This mechanism can be looked upon as a significant pathway. The incidence of red light begins photosynthetic process and this is when the plant will need CO_2 the most. To meet this co_2 requirement the stomata also need to open maximally.
6. The charge asymmetry that results in the guard cell because of H^+ extrusion is balanced by passive intake of K^+ ions. The driving force for this passive intake is the electrical potential gradient created by H^+ extrusion.
7. K^+ ions activate Chloride channels. Thus, Cl^- comes inside the guard cell.
8. Accumulation of K^+ and Cl- ions inside the guard cells bring down the levels of ψ_s inside the cell. This results into osmotic uptake of water from the neighbouring cells.
9. This uptake of water increases the turgidity of the stomatal cell that leads to curved stretching out of the cell and eventual stomatal opening.

Additional role of the red light in guard cell osmoregulation

Recent studies of daily courses stomatal behaviour in many plants shows that K^+ ions accumulate in the guard cells in the morning and early noon. By the afternoon potassium ion concentration starts dissipating, yet stomata remains open. They close only towards the evening.

Further, it has been shown that sucrose starts accumulating since the morning, builds up through the day, and dissipates only in the evening. Sucrose is also a highly active osmotic solute.

It has been concluded that K^+ ions are responsible for rapid stomatal opening in the morning but the maintenance of the opening is done by sucrose build-up. Stomatal closure is thus mediated by sucrose dissipation in the evening.

The source of sucrose is currently not clear and it seems to vary with the species. Sucrose may come from:

- Starch hydrolysis induced by red light. In this reaction, Phytochrome is the photoreceptor.
- Apoplastic uptake of sucrose synthesized by mesophyll cells
- Guard cells own photosynthesis, in some species.

Other important organic solute that builds up in a number of plants is Malate^{2-}. This ion is synthesized in the guard cell cytoplasm itself rather than being imported. A possible pathway of malate^{2-} build up is as follows:

Starch → Phosphoenol pyruvate [PEP] + CO_2 → Oxaloacetate → Malate^{2-}.

Stomatal closure

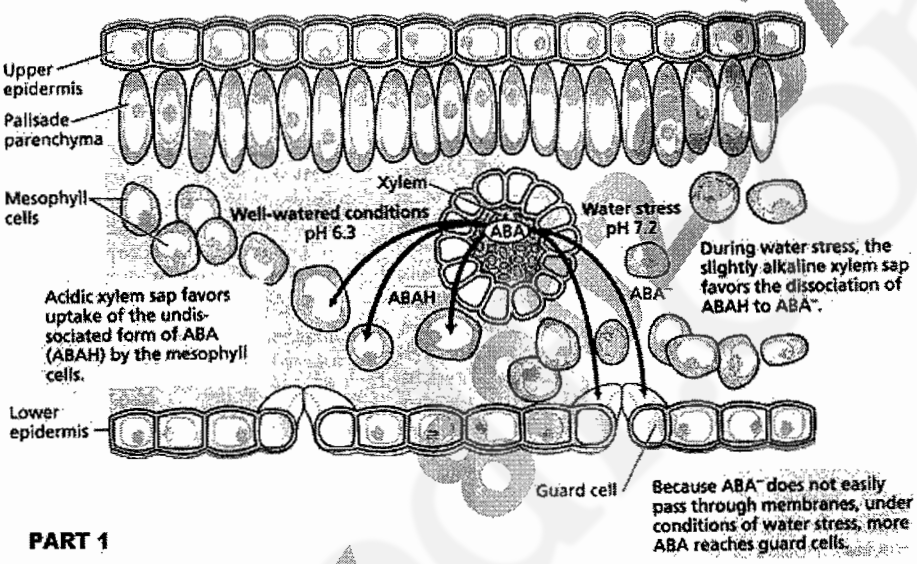
In the evening when photosynthesis ceases and there is no longer a need for CO_2:

- Sucrose converts into starch, or
- May get exported out of the guard cells

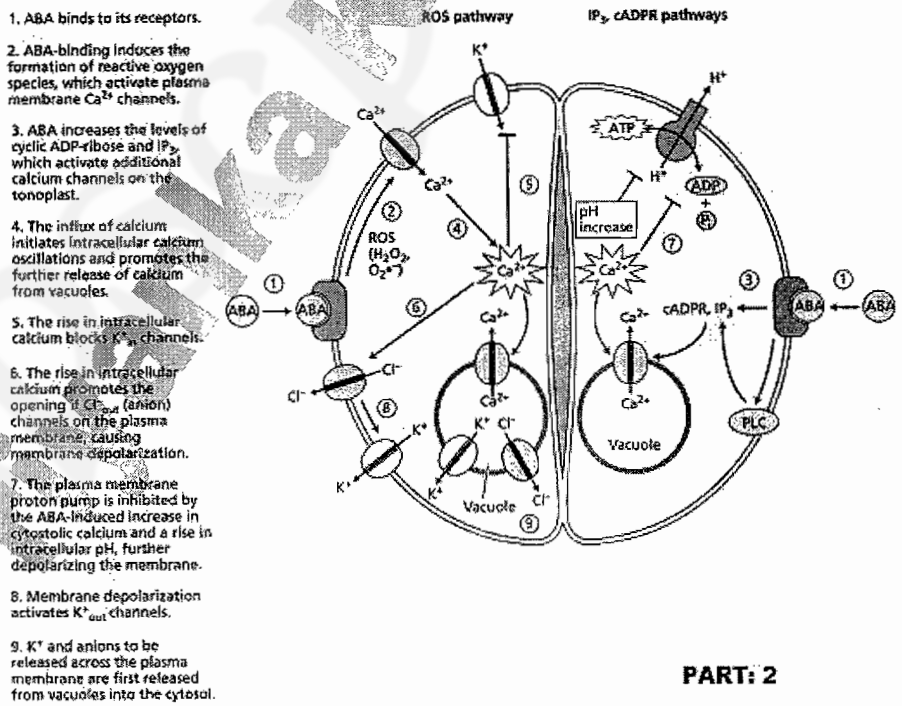
- K^+ and Cl^- ions have already dissipated in the afternoon.
- $Malate^{2-}$ is converted back into starch.

All these factors lead to rising up of ψ_w in the guard cell. This results into EXOSMOSIS OF WATER FROM THE GUARD CELL and ultimately it becomes flaccid. Thus, the guard cells come back to their non-turgid shape and the stomatal pores close.

During water stress conditions, the stress hormone ABA causes forced closure of stomata through the following process.



PART 1



PART: 2

Mineral nutrition and ion transport, mineral deficiencies

Introduction

Of the naturally occurring 92 elements of the periodic table, about 20 are essential to plants. Water and CO_2 provide the plant with the elements C, H and O; the remaining necessary elements are obtained by flowering plants as inorganic mineral ions, mostly from the soil solution.

Essential elements

An element is classed as essential to a plant if:

1. The plant cannot complete its life cycle without it.
2. There is no other element can substitute for it.
3. The effect of the element must also be direct, i.e. it should not act by promoting the uptake of another essential element, or by retarding the absorption of a toxic one.

To test for the essentiality of an element, the test plants must be placed in an environment totally free from that element. This means growing the plants in a liquid culture medium of precisely known composition.

In addition to essential elements there are **beneficial elements**. They are not absolutely necessary for survival but promote the growth and vigour of plants.

Non-essential elements are also taken up by plants; any element present in the environment will be absorbed at least in small amounts. For plants grown in the soil, large amounts of Al and Na are frequently present as these are common in soils. Though inessential, such elements are not inert. They influence the ionic balance and osmotic potential of the cells and may affect the uptake of essential ions.

Many non-essential elements are toxic also and their uptake is detrimental to the plants and to the animals which feed on them.

Macronutrients and Micronutrients

The essential elements are classified as macronutrients and micronutrients.

1. **The macronutrients are** required in large amounts relative to the micronutrients. In culture solutions, macronutrients are supplied at 10^{-3} to 10^{-2} mol L^{-1}.
2. **The micronutrients** are required in concentrations as low as 10^{-7} mol L^{-1}. Most of the micronutrients become toxic at quite moderate concentrations, that is above 10^{-4} mol L^{-1}.

Element	Symbol	Form absorbed
Essential macronutrients		
1. Carbon	C	CO_2, CO_3^{2-} (carbonate), HCO_3^- (bicarbonate)
2. Hydrogen	H	H_2O
3. Oxygen	O	O_2, H_2O, CO_2
4. Nitrogen	N	NO_3^- (nitrate), NH_4^+ (ammonium)
5. Sulphur	S	SO_4^{2-} (sulphate)
6. Phosphorus	P	$H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-} (phosphates)
7. Calcium	Ca	Ca^{2+}
8. Potassium	K	K^+
9. Silicon*	Si	H_4SiO_4 (silicic acid)
Essential micronutrients		
10. Iron	Fe	Fe^{2+} (ferrous)
11. Magnesium	Mg	Mg^{2+}
12. Manganese	Mn	Mn^{2+}
13. Copper	Cu	Cu^{2+} (cupric)
14. Zinc	Zn	Zn^{2+}
15. Boron	B	H_3BO_3 (boric acid)
16. Nickel	Ni	Ni^{2+}
17. Cobalt*	Co	Co^{2+}
18. Molybdenum*	Mo	MoO_4^{2-} (molybdate)
19. Chlorine*	Cl	Cl^- (chloride)
20. Sodium*	Na	Na^+

Element	Symbol	Form absorbed
Beneficial elements		
Selenium	Se	SeO_4^{2-} (selenate)
Rubidium	Rb	$Rb+$
Strontium	Sr	Sr^{2+}
Aluminium	Al	Al^{3+}

Note: An asterisk* marked element has so far been found to be essential only in some species; of these, silicon, chlorine and sodium are *beneficial* in numerous other species.

Role of essential elements in plant physiology

Element	Role in plant physiology
Carbon	Carbon, hydrogen and oxygen are constituents of all organic molecules and the majority of organic molecules of living cells. They are most abundant in the plant biomass.
Hydrogen	
Oxygen	
Nitrogen	Nitrogen, too, is a constituent of many cellular molecules, in particular proteins and nucleic acids, the key macromolecules of life. There are many lower molecular weight nitrogenous organic compounds vital to cell metabolism - vitamins, cofactors, hormones, the chlorophyll pigments and the phytochrome photoreceptors. Flowering plants additionally contain a variety of nitrogenous secondary compounds not involved in basic metabolism. These include alkaloids. Plants also contain numerous non-protein amino acids, which are not incorporated into normal proteins. Both the alkaloids and the non-protein amino acids are toxic and often bitter tasting; and one possible function is protection against herbivores. In seeds, non-protein amino acids, with a high proportion of N by weight, can act as N storage compounds. Some non-photosynthetic pigments contain N, e.g. betacyanin, the red pigment of beetroot (*Beta vulgaris*).
Sulphur	Sulphur performs an important structural role in proteins where the disulphide bridges -S-S- stabilize tertiary protein structures. Sulphydryl

Element	Role in plant physiology
	groups, -SH, are found in the active sites of many enzymes. There are also -SH-containing coenzymes, e.g. coenzyme A, whilst glutathione, again with a -SH group, is an important antioxidant. Several iron-sulphur proteins, e.g. ferredoxins, occur in the electron transfer systems of chloroplasts and mitochondria; these proteins contain clusters of linked S and Fe atoms at their reactive sites. Membrane sulpholipids are structural molecules which contain a sulphate group, found in chloroplast thylakoid membranes. Numerous flowering plants contain pungent secondary S-containing compounds appreciated as flavours; these are very common in the Brassicaceae (cabbage family) which includes mustard (*Sinapis alba*). Onions (*Allium cepa*), garlic (*Allium sativum*) and related species are also flavoured with S-containing chemicals. The presence of such compounds may deter some herbivores.
Phosphorus	Phosphorus is contained in nucleic acids and also in membrane phospholipids which make up the bimolecular lipid leaflet of biological membranes. As a component of the adenosine phosphates (ATP, ADP and AMP) and related nucleotides, the phosphate group is involved in 'energy metabolism'.
Calcium	Calcium contributes to membrane stability in plant cells by its association with membrane phospholipids, and it is necessary for the maintenance of the normal permeability of the plasmalemma. In plants it also contributes to cell wall structure as calcium pectate; this is a major component of the middle lamella which cements adjacent cell walls together. The Ca^{2+} ion is extremely important in stimulus perception; one of the first effects in the chain of reactions set off by a stimulus, environmental or hormonal. Ca^{2+} is also termed a 'second messenger'.
Potassium	It is present in cells as the free K+ ion; it does not enter into organic combination. It is known to be the activator of some enzymes. K^+ ions are concerned in turgor control; such cells are stomatal guard cells and the pulvinar cells (hinge cells) of leaves and petioles.

Element	Role in plant physiology
Silicon	Silicon in the form of silica gel, a hydrated oxide of Si, gives the cell walls of grasses, including cereals, their characteristic rigidity; this is very conspicuous in the dried-out straw. Si is not known to take part in any biochemical reactions within cells.
Iron	Iron and copper are present in the respiratory and photosynthetic electron transfer chain cytochromes. They are also needed for other oxidative enzymes: Fe for catalase and peroxidase, Cu for ascorbic acid oxidase and polyphenol oxidase; Fe is present in iron-sulphur proteins, as mentioned in connection with S . Fe is also necessary for chlorophyll synthesis.
Copper	
Magnesium	Magnesium and manganese activate many dehydrogenases and phosphate transfer enzymes and are also important in photosynthesis, a Mg atom being part of the chlorophyll molecule whereas Mn is present in the O_2-evolving complex.
Manganese	
Zinc	Zinc is an activator for many enzymes. Particularly important in plants are alcohol dehydrogenase, superoxide dismutase (which degrades the highly reactive and dangerous superoxide radicals formed during certain oxidative and photosynthetic reactions), and carbonic anhydrase.
Boron	Boron is the element for which the physiological role has proved most difficult to elucidate. Much of the B in the plant is associated with cell walls where it cross-links cell wall polymers, such as pectins. It also is needed for normal membrane function. In B-deficient roots, ion uptake capacity deteriorates but when such roots are supplied with B, recovery is considerable by 20 minutes and complete within an hour.
Nickel	Nickel is a constituent of the enzyme urease, which hydrolyses urea; the enzyme is needed for N metabolism in plants.
Cobalt	Cobalt also is needed in minute quantities only, and is known to be needed for symbiotic N_2 fixation, which involves the Co- containing vitamin B_{12}.
Molybdenum	Molybdenum is present in the enzyme nitrate reductase, which is needed to utilize nitrate, the major source of inorganic N for most plants, and it is needed for symbiotic N_2 fixation. It is also part of the cofactor (Moco) for aldehyde oxidase, an enzyme involved in the synthesis of ABA, and in a few other oxidases. The amount required is extremely small.

Element	Role in plant physiology
Chlorine	Chlorine is required for the O_2-evolving system of photosynthesis. For this it is needed in only micronutrient amounts. However, the element is taken up by cells in large quantities and the chloride ion Cl^- is the chief inorganic anion in cells, often accompanying K+, e.g. during K+ fluxes in stomatal guard cells, so that it is beneficial in much larger amounts than required to fulfil its essential biochemical role.
Sodium	Sodium is required for C_4 photosynthesis in some C_4 species where it seems to be involved with the conversion of pyruvate to PEP. It is present in cells as the free Na+ ion and like Cl^- is tolerated in relatively high concentrations. Chemically it is very similar to K and to some extent it can interchange with that element; e.g. in ***Commelina benghalensis*** Na can replace K in the control of turgor of the stomatal guard cells. In succulent halophytes, plants which live in saline habitats, Na+ acts as an osmoregulatory ion, with Cl^-.

Important deficiency symptoms

Symptoms caused by nutrient deficiencies are generally grouped into five categories: 1) stunted growth, 2) chlorosis, 3) interveinal chlorosis, 4) purplish-red coloring and 5) necrosis.

Nitrogen (N)

Function:-Nitrogen is the most critical nutrient for optimum farm production. Since it is readily leached from the soil, its level in soil is typically low, however the levels required for optimum crop growth are quite high and thus generous application is typically required. Nitrogen reduces all aspects of crop and pasture production e.g. growth, yield and quality.

Symptoms of Deficiency :-Plants which have Nitrogen for only limited growth, may exhibit chlorosis especially in the older leaves. In severe cases, the leaves first yellow and then tan as they die. Some plants (tomatoes, maize) may exhibit a purplish colouration of the stems, petioles and on the underside of their leaves .

Phosphorous (P)

Function:-Phosphorous is an essential element for cell division and growth. It is required for photosynthesis, sugar and starch production, energy transfer and the movement of carbohydrates within the plant and reproduction.

Symptoms of Deficiency:-Plants exhibit stunted growth and leaves are often dark green in colour.Oldest leaves become dark brown as they die. Maturity may be delayed.

Potassium (K)

Function:-Potassium is an essential element for protein, carbohydrate and fat synthesis and is required for the proper functioning of chlorophyll and other enzymes involved in photosynthesis,respiration and protein formation.It is essential for cell division, cell electrolyte balance and for the functioning of plant stomates.

Symptoms of Deficiency:-Crops suffering this deficiency appear scorched around the edges and surfaces are irregularly chlorotic. In legumes, the chlorotic spots form patterns around the leaf edges. Cereal grains develop weak stalks and their roots may become more prone to infection by root rotting organisms.These two factors may result in collapse of the crop by wind or rain.

Iron (Fe)

Function:-Iron is an essential element required for the synthesis of chlorophyll. It is involved in the activation of many enzymes used in photosynthesis and respiration.

In animals, iron is a key constituent of haemoglobin, the species that carries oxygen in the blood.

Iron is relatively immobile and is generally in short supply in alkaline soils.

Symptoms of Deficiency:-Young leaves develop chlorosis in the interveinal areas which may develop into white leaves with necrotic spots.Stunted growth.

Manganese (Mn)

Function:-Manganese is an essential nutrient for the growth of both plants and animals. In plants it enhances root growth, disease resistance and the development of fruit. It is required for the synthesis of chlorophyll and assimilation of nitrate. It is involved in the activation of many enzymes involved in photosynthesis and respiration.

In animals, manganese is essential for growth, reproduction, skeletal growth and carbohydrate metabolism.

Symptoms of Deficiency:-Symptoms vary with species; in cereals – grey – white spots, flecks and stripes may appear in the interveinal areas. In legumes, interveinal chlorosis of young and middle aged leaves and tissue may rapidly become necrotic. Seed disorders e.g. "split seed" or "marsh spot" may develop.

Boron (B)

Function:-Boron is an essential element in plant nutrition. It is essential for root tip, pollen tube and shoot growth and the synthesis of DNA and RNA.

Symptoms of Deficiency:-Leaf blades may be distorted and stems may become brittle and crack e.g. "stem crack" in celery. Shorter intermodal length, retarded growth or necrosis of the terminal buds and youngest leaves. Reduction or failure to seed and fruit. Malformation of fruit.

Zinc (Zn)

Function:-Zinc is an essential nutrient required for the functioning of a large number of enzymes involved in the growth and reproduction of both plants and animals. It is required for the synthesis and functioning of chlorophyll, is involved in the plant hormone system and as a catalyst for the plant growth regulator, auxin.

Symptoms of Deficiency:-In plants, shortened internodes with excessive branching (resetting) of small, dark green deformed leaves. In cereals and grasses – chlorotic bands (yellow, red) may appear either across or within the veins. Stunted growth and necrosis of older leaves.

Copper (Cu)

Function:-Copper is an essential nutrient required for the functioning of a large number of enzymes involved in the growth and reproduction. It is required for the synthesis and functioning of chlorophyll, is involved in the plant hormone system and acts as a catalyst for the plant growth regulator, auxin.

Symptoms of Deficiency:-Young leaves become dark green,twisted and deformed. Necrotic spots may appear. In grains and grasses, seed production is reduced and seed heads may be white and empty.

Molybdenum (Mo)

Function:-Molybdenum is an essential element for both plants and animals. In plants, Molybdenum is required for protein synthesis.It enhances both photosynthesis in plants and nitrogen fixation in legumes. In animals, Molybdenum is a constituent of several important enzymes, and plays a role in animal fertility, the estrus cycle, and mammary anticarcinogenesis.

Symptoms of Deficiency:-In plants, reduced and irregular leaf blade formation, interveinal mottling and chlorosis around the edges of older leaves. Necrotic spots at leaf tips and edges, smaller root nodules coloured white or green (not pink), growth inhibition in legumes.

Cobalt (Co)

Function:-Cobalt is a key constituent of Vitamin B12 and Propionate (themajor source of energy in ruminants). Cobalt enhances thenitrogen fixing ability of legumes and improves the efficiency of ruminal digestion.

Symptoms of Deficiency:-Small root nodules on legume species.

Uniformly pale green – yellow leaves,most severe on old leaves. Some crops may develop red leaves, stems or petioles. Stunted growth – tops may be less leafy. Grain or seed production may be retarded.

Magnesium (Mg)

Function:-Magnesium is an essential nutrient for the synthesis of chlorophyll. It is involved with the functioning of several enzymes associated with photosynthesis,respiration and reproduction.

Symptoms of Deficiency:-Interveinal chlorosis of older leaves.

Mineral uptake from the soil by plant roots

With the exception of C, H and O, which are derived from water and CO_2 and are incorporated by photosynthesis, plants acquire all other elements as inorganic ions.

The ions which serve as sources of the essential elements for flowering plants are listed in a table earlier. For terrestrial flowering plants the chief source of mineral ions is the soil. The mineral rock particles of the soil yield ions by weathering which gradually brings them into solution; ions are also released by the action of microorganisms on dead organic material. The ion concentration of the soil solution rises as the water content of the soil falls, but except under very dry conditions the solution is very dilute.

Not all the ions in the soil are totally free in the soil solution. The colloidal matter in the soil, both inorganic clay particles and organic particles 'humus' also serve to retain ions by adsorption.

The colloidal constituents of the soil usually carry a net negative charge; cations, being positively charged, are adsorbed to the negatively charged groups on the clay and on the organic particles. These ions are held at the surface of the soil particles by electrostatic attraction only loosely and **can be exchanged for other cations**.

Plants release H^+ ions in the soil which can displace many cations from the surface of soil particles and make them available in the soil solution freely.

There are two mechanisms of increasing H^+ concentration is the soil.

1. Active pumping of protons by the root cells
2. Release of CO_2 as a result of respiration increases the levels of H_2CO_3 in the soil ($CO_2 + H_2O \rightarrow H_2CO_3$). H_2CO_3 then dissociates into HCO_3^- and H^+ ions.

When in the soil solution, the levels of H^+ ions increase, the essential cations become available freely which can then be taken by the root cells **mostly by active transport**.

Essential anions are usually available freely in the soil sap, therefore they can be taken up directly using a suitable membrane transport method.

The long-distance transport of ions takes place in the xylem concurrently with water transport.

Just as for water, the ions move by an apoplastic, symplastic or transcellular route

Apoplastic ion movement is partly by diffusion, along with the flow of water moving into the transpiration stream.

The endodermal Casparian strips are believed to form a barrier to ion movement, as for water. Here, the ionic movement becomes symplastic.

Once in the root xylem, the mineral ions move up into the aerial parts with the xylary stream of sap. (Please refer to the earlier coverage on **Ascent of Sap**).

Role of Mycorrhiza

The word mycorrhiza means 'fungus-root'. It is the name given to a symbiotic association between a plant root and a fungus, which in most cases enhances the mineral nutrient supply of the plant, whilst the fungus benefits from a supply of organic C from the plant.

Mycorrhizal associations occur in most species of flowering plants in the field and it is highly beneficial.

In a mycorrhizal association, part of the fungal mycelium is free in the soil, part is closely associated with roots.

Exchange of nutrients takes place over the large area of contact between the fungus and the root cells.

The surface area of fungus exposed to the soil is also very large, enabling efficient mineral absorption.

Phloem Transport

Introduction to Phloem

In vascular plants, **phloem** is a complex living tissue that carries organic nutrients, particularly sucrose, amino acids, fatty acids, certain signaling factors and a few RNA molecules to all parts of the plant where needed.

Phloem tissue consists of less specialized and nucleate *parenchyma* cells, ***sieve-tube cells***, and ***companion cells*** (in addition to fibres and *sclereids*) (Fig. 1).

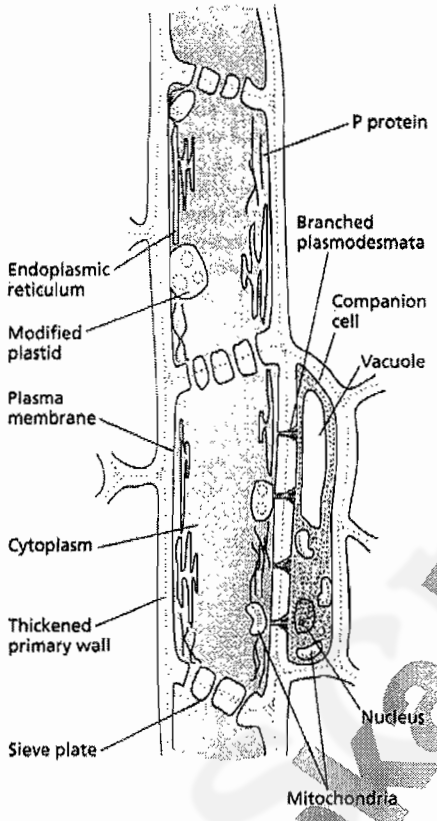


Figure 1: Phloem Structure in Angiosperms

Sieve tube element

The sieve-tube element cells lack a nucleus, have very few vacuoles, but contain other organelles such as ribosomes. The endoplasmic reticulum is concentrated at the lateral walls. Sieve-tube members are joined end to end to form a tube that conducts food materials throughout the plant. The end walls of these cells have many small pores and are called sieve plates and have enlarged plasmodesmata.

Companion cells

The survival of sieve-tube members depends on a close association with the *companion cells*. All of the cellular functions of a sieve-tube element are carried out by the (much smaller) companion cell, a typical plant cell, except the companion cell usually has a larger number of ribosomes and mitochondria. This is because the companion cell is more metabollically active than a 'typical' plant cell. The cytoplasm of a companion cell is connected to the sieve-tube element by plasmodesmata.

There are three types of companion cell.

1. **Ordinary companions cells** - which have smooth walls and few or no plasmodesmata connections to cells other than the sieve tube.
2. **Transfer cells** - which have much folded walls that are adjacent to non-sieve cells, allowing for larger areas of transfer. They are specialised in scavenging solutes from those in the cell walls which are actively pumped requiring energy.
3. **Intermediary cells** - which have smooth walls and numerous plasmodesmata connecting them to other cells.

The first two types of cell collect solutes through apoplastic (cell wall) transfers, whilst the third type can collect solutes symplastically through the plasmodesmata connections.

In gymnosperms, the sieve tube element is not found. Instead, they contain Sieve Cells. The difference between Sieve Tube Elements and Sieve Cells is given below.

Sieve tube elements found in angiosperms

1. Some sieve areas are differentiated into sieve plates; individual sieve tube elements are joined together into a sieve tube.
2. Sieve plate pores are open channels.
3. P-protein is present in all dicots and many monocots.
4. Companion cells are sources of ATP and perhaps other compounds and, in some species, are transfer cells or intermediary cells.

Sieve cells found in gymnosperms

1. There are no sieve plates; all sieve areas are similar.
2. Pores in sieve areas appear blocked with membranes
3. There is no P-protein.
4. Albuminous cells sometimes function as companion cells.

Patterns of Translocation

Sap in the phloem is not translocated exclusively in either an upward or a downward direction, and translocation in the phloem is not defined with respect to gravity. Rather, sap is translocated from areas of supply, called sources, to areas of metabolism or storage, called sinks.

Sources include any exporting organs, typically mature leaves, that are capable of producing photosynthate in excess of their own needs. The term photosynthate refers to products of photosynthesis. Another type of source is a storage organ during the exporting phase of its development. For example, the storage root of the biennial wild beet (Beta maritima) is a sink during the growing season of the first year, when it accumulates sugars received from the source leaves. During the second growing season the same root becomes a source; the sugars are remobilized and utilized to produce a new shoot.

Sinks include any nonphotosynthetic organs of the plant and organs that do not produce enough photosynthetic products to support their own growth or storage needs. Roots, tubers, developing fruits, and immature leaves, which must import carbohydrate for normal development, are all examples of sink tissues.

Essentials of Phloem Translocation

Translocation in the phloem is the movement of the products of photosynthesis from mature leaves to areas of growth and storage. The phloem also redistributes water and various compounds throughout the plant body.

Some aspects of phloem translocation have been well established by extensive research over many years. These include the following:

- *The pathway of translocation*. Sugars and other organic materials are conducted throughout the plant in the phloem, specifically in cells called sieve elements. Sieve elements display a variety of structural adaptations that make them well suited for transport.

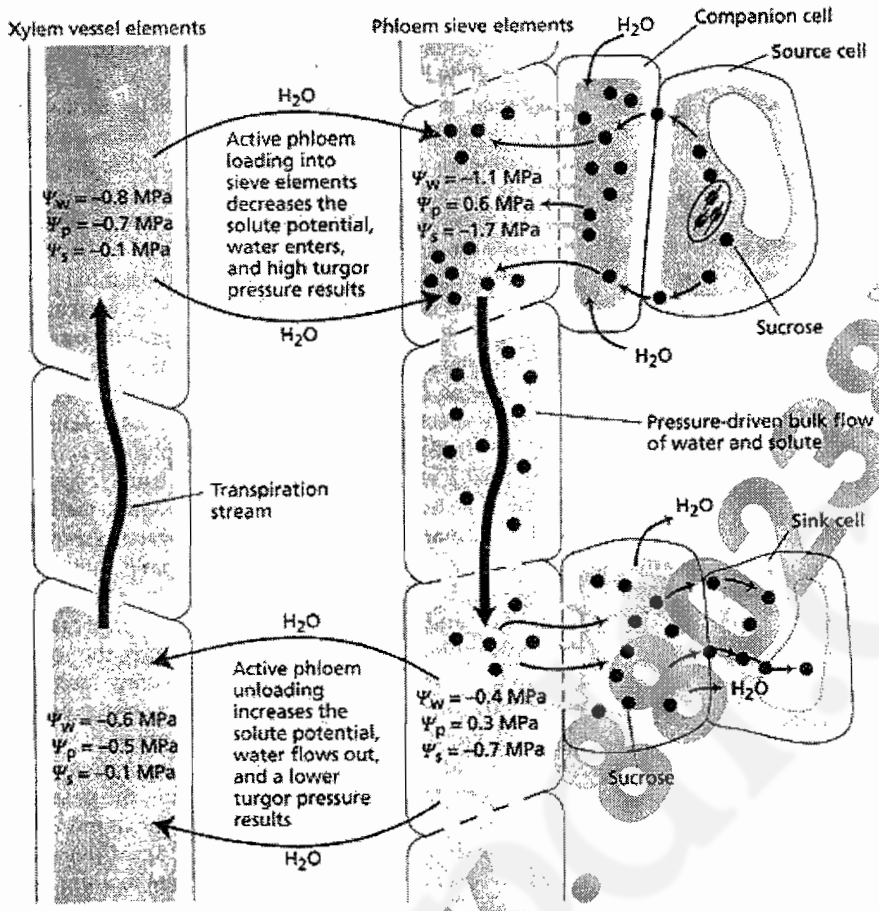
- *Patterns of translocation*. Materials are translocated in the phloem from sources (areas of photosynthate supply) to sinks (areas of metabolism or storage of photosynthate). Sources are usually mature leaves. Sinks include organs such as roots and immature leaves and fruits.
- *Materials translocated in the phloem*. The translocated solutes are mainly carbohydrates, and *sucrose is the most commonly translocated sugar*. Phloem sap also contains other organic molecules, such as amino acids, proteins, and plant hormones, mRNA, as well as inorganic ions.
- *Rates of movement*. Rates of movement in the phloem are quite rapid, well in excess of rates of diffusion. Velocities average 1 m h^{-1}, and mass transfer rates range from 1 to 15 g h^{-1} cm^{-2} of sieve elements.

Mechanism of Phloem Translocation

The best-supported theory to explain the movement of food through the phloem is called the **pressure-flow hypothesis (PFH)**. This hypothesis was proposed by Ernst Munch in 1930. In this model the bulk flow of phloem sap occurs in response to an osmotically generated pressure gradient. A variety of structural and physiological data indicate that materials are translocated in the phloem of angiosperms by pressure flow. The mechanism of translocation in gymnosperms requires further investigation.

The essentials of PFH

- It proposes that water containing organic material flows under pressure through the phloem. The pressure is created by osmotic buildup of water due the difference in water potential of the solution in the phloem and the relatively pure water in the nearby xylem ducts. The water potential of the solution in the phloem is lower than the xylary water (Fig. 2).
- The low water potential of the solution in the phloem is created by active transport sugars into the companion cells and sieve elements of the phloem. As sugars (and other products of photosynthesis) accumulate in the phloem, water enters by osmosis from neighbouring xylem tissue.
- Turgor pressure builds up in the sieve tube.
- As the fluid is pushed down (and up) the phloem, sugars are removed by the cortex cells of both stem and root (the "sinks") and consumed or converted into starch. Starch is insoluble and exerts no osmotic effect. Therefore, the osmotic pressure of the contents of the phloem decreases.
- Finally, relatively pure water is left in the phloem, and this leaves by osmosis and/or is drawn back into nearby xylem vessels by the suction of transpiration-pull.
- Thus, it is the pressure gradient between "source" (leaves) and "sink" (shoot and roots) that drives the contents of the phloem up and down through the sieve tubes.



Schematic diagram showing the pressure-flow model of translocation in the phloem. At the source, sugar is actively loaded into the sieve element–companion cell complex. Water enters the phloem cells osmotically, building up a high turgor pressure. At the sink, as sugars are unloaded, water leaves the phloem cells and a lower pressure results. Water and its dissolved solutes move by bulk flow from the area of high pressure (source) to the area of low pressure (sink). Possible values for Ψ_w, Ψ_p, and Ψ_s in the xylem and phloem are illustrated. Note that this illustration shows sieve element loading as apoplastic and sieve element unloading as symplastic with a later apoplastic step; this scenario is certainly not universal. See the sections in the text on loading and unloading. (After P. S. Nobel 1991.)

Phloem loading and unloading

Transport of sugars into and out of the sieve elements is called sieve element loading and unloading, respectively. In some species, sugars must enter the apoplast of the source leaf before **Active Loading**. In these plants, loading into the sieve elements requires metabolic energy, provided in the form of a proton gradient. Some plants however appear not to load phloem by active transport. In these cases a mechanism known as the **Polymer Trap Mechanism** was proposed by Robert Turgeon in 1991. In this case, small sugars such as sucrose move into intermediary cells through narrow plasmodesmata, where they are polymerised to raffinose and other larger oligosaccharides. Now they are unable to move back, but can proceed through wider plasmodesmata into the sieve tube element. The polymer trap mechanism is confined mostly to plants in tropical rain forests and is seen as more primitive. The actively-transported apoplastic phloem loading is viewed as more advanced, as it is found in the later-evolved plants, and particularly in those in temperate and arid conditions. This mechanism may therefore have allowed plants to colonise the cooler locations.In either case, phloem loading is specific for the transported sugar.

Phloem unloading requires metabolic energy, but the transport pathway, the site of metabolism of transport sugars, and the site where energy is expended vary with the organ and species.

Support for the PFM

1. The contents of the sieve tubes must be under pressure. This is difficult to measure because when a sieve tube is punctured with a measuring probe, the holes in its end walls quickly plug up. However, aphids can insert their mouth parts without triggering this response. When it punctures a sieve tube, sap enters the insect's mouth parts under pressure and some soon emerges at the other end (as a drop of honeydew that serves as food for ants and bees). Honeydew will continue to exude from the mouthparts after the aphid has been cut away from them.
2. The osmotic pressure of the fluid in the phloem of the leaves must be greater than that in the phloem of the food-receiving organs such as the roots and fruits. Most measurements have shown this to be true.

Nitrogen fixation and nitrogen metabolism

Nitrogen fixation is the process by which nitrogen is taken in its molecular form (N_2) in the atmosphere and converted into compound form such as ammonia, nitrate and nitrogen dioxide.

It is a very important process in the biosphere because of two reasons.

1. Nitrogen is an essential element for all organisms being a part of amino acids, proteins, nucleic acids, coenzymes, vitamins and other cellular constituents etc.
2. Most organisms can use nitrogen only in compound form. Only a few prokaryotes, known as Diazotrophs can use nitrogen in its molecular form, i.e. N_2.

Types of nitrogen fixation

Three processes are responsible for most of the nitrogen fixation in the biosphere:

1. Atmospheric fixation by lightning
2. Biological fixation by certain microbes – free living or in a symbiotic relationship with plants
3. Industrial fixation by Haber and Bosch Process

Atmospheric Fixation

The enormous energy of lightning breaks nitrogen molecules and enables their atoms to combine with oxygen in the air forming nitrogen oxides. These dissolve in rain, forming nitrates which are carried to the earth.

Atmospheric nitrogen fixation probably contributes some 5– 8% of the total nitrogen fixed.

Industrial Fixation by Haber & Bosch Process

Under 15–25 MPa (150–250 bar) pressure, at a temperature of 600°C, and with the use of a catalyst, atmospheric nitrogen and hydrogen (usually derived from natural gas or petroleum) can be combined to form ammonia (NH_3). Ammonia can be used directly as fertilizer, but most of it is further processed to urea and ammonium nitrate (NH_4NO_3).

Biological Fixation

The ability to fix nitrogen is found only in certain bacteria and cyanobacteria known as Diazotrophs.

- Some live in a symbiotic relationship with plants of the legume family (e.g., soybeans, alfalfa).
- Some establish symbiotic relationships with plants other than legumes (e.g., alders).
- Most nitrogen-fixing bacteria live free in the soil.
- Nitrogen-fixing cyanobacteria mostly live in semi-aquatic environments like paddy fields.

Biological nitrogen fixation requires Nitrogenase complex enzyme and a large expenditure of ATP. Although the first stable product of the process is ammonia, this is quickly incorporated into protein and other organic nitrogen compounds. The following table shows relative contribution of various agents of nitrogen fixation in the biosphere.

The Nitrogen Fixing Organisms

Free Living Nitrogen Fixers: Aerobes

1. **Cyanobacteria**

Anabaena, Nostoc, Gloeotheca, Calothrix

2. **Chemoorganotrophs**

Azotobacter, Azomonas, Agrobacterium, Klebsiella (Facultative), *Beijerinckia, Bacillus polymyxa* (Facultative), *Azospirillum, Citrobacter, Methylomonas, Methylococcus, Pseudomonas, Derxia*

3. **Chemolithotrophs**

Alcaligens, Thiobacillus, Acidithiobacillus,Streptomyces thermoautotrophicans

Free Living Nitrogen Fixers: Anaerobes

1. **Chemoorganotrophs**

Clostridium, Desulfovibrio, Desulfobacter, Desulfotomaculum

2. **Phototrophs**

Chromatium, Ectothiorhodospira, Thiocapsa, Chlorobium, Chlorobaculum, Rhodospirillum, Rhodopseudomonas, Rhodomicrobium, Rhodopila, Rhodobacter, Heliobacterium, Heliobacillus, Heliophilum

3. **Archaea**

Methanococcus, Methanosarcina, Methanobacterium, Methanospirillum, Methanolobus

Symbiotic Nitrogen Fixers: Non Leguminous Host

1. *Frankia*: Wide host range in many tree species of angiosperms, such as *Alnus, Casuarina, Colletia, Coriaria, Myrica, Discaria, Rubus, Cowania, Purshia* and some shrubs like, *Datisca, Coenothus* and *Cercocarpus ledifolius.*
2. *Acetobacter*: Sugar cane
3. *Nostoc*: the hornwort *Anthoceros*, the liverwort *Porella*, the lichen *Lobaria pulmonaria*
4. *Anabaena*: the fern *Azolla*
5. *Nostoc*: *Gunnera* (forming petiolar nodules)
6. *Nostoc*: The palm *Welfia regia*
7. *Nostoc* + *Anabaena*: the coralloid roots of *Cycas, Ceratozamia mexicana*

Symbiotic Nitrogen Fixers: Leguminous or some closely related Host

By 5 genera of closely related diazotrophs: *Rhizobium, Azorhizobium, Bradyrhizobium, Photorhizobium, Sinorhizobium*

Host	Bacterium
Parasponia (a non legume, formerly called *Trema*)	*Bradyrhizobium sp.*
Glycine max (Soybean)	*Bradyrhizobium japonicum* (Slow Infection), *Sinorhizobium fredii* (Fast Infection)
Medicago sativa (Alafalfa), *Melilotus*	*Sinorhizobium meliloti*
Sesbania	*Azorhizobium* (forming leaf and adventitious root nodules)
Phaseolus	*Rhizobium leguminosarum* bv. *phaseoli* *Rhizobium tropicii* *Rhizobium etli*
Trifolium (Clovers)	*Rhizobium leguminosarum* bv. *trifolii*
Pisum sativum, Lathyrus, Lens, Vicia faba	*Rhizobium leguminosarum* bv. *viciae*
Lotus	*Rhizobium loti*
Aschenomene	*Photorhizobium* (forming leaf and adventitious root nodules)

The Biochemistry of Nitrogen Fixation

Whether symbiotic or free living, the enzymology and biochemistry of Nitrogen Fixation is the same. Some bacteria, called Diazotrophs, convert N_2 into ammonia by the enzyme termed Nitrogense Complex. Biological nitrogen fixation can be represented by the following equation, in which two moles of ammonia are produced from one mole of nitrogen gas, at the expense of 16 moles of ATP and a supply of 8 electrons and 8 protons.

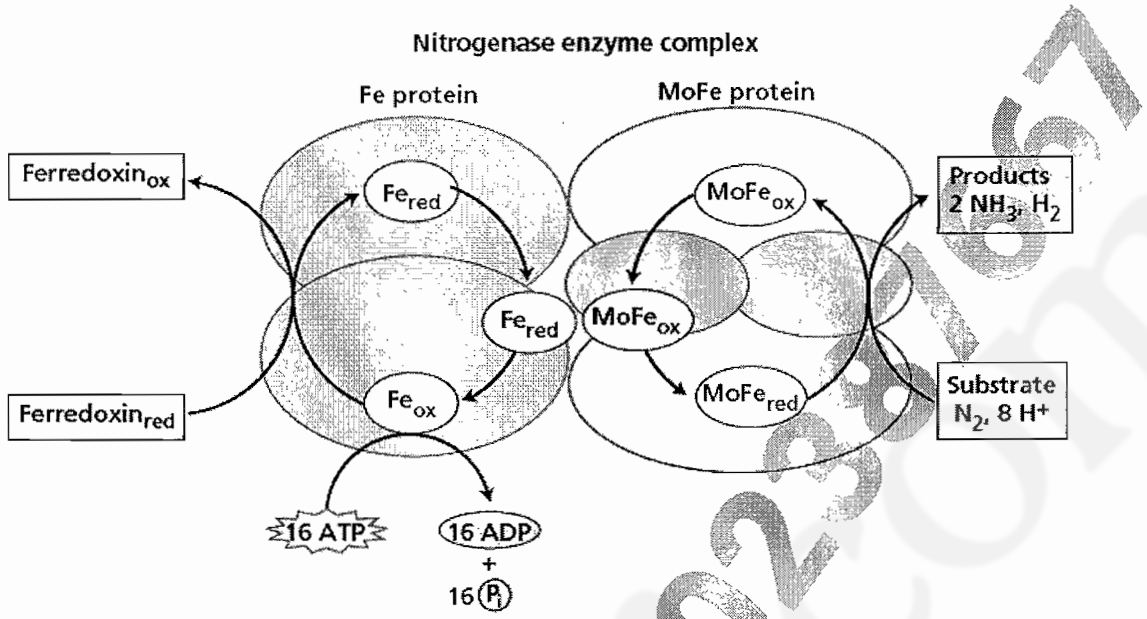
$N_2 + 8H+ + 8e^- + 16\ ATP = 2NH_3 + H_2 + 16ADP + 16\ Pi$

This reaction is performed exclusively by prokaryotes (the bacteria and related organisms).

The reactions occur while N_2 is bound to the nitrogenase enzyme complex. The steps are as follows:

1. The Fe protein is first reduced by electrons donated by ferredoxin.
2. Then the reduced Fe protein binds ATP and reduces the molybdenum-iron protein,
3. The reduced the molybdenum-iron protein donates electrons to N_2, producing HN=NH. In two further cycles of this process $2NH_3$ is produced.

Depending on the type of microorganism, the reduced ferredoxin which supplies electrons for this process is generated by photosynthesis, respiration, fermentation or Pentose Phosphate pathway, in case of some symbioses.



Structure of the Nitrogenase Complex

There is a remarkable degree of functional conservation in the nitrogenase proteins of all nitrogen-fixing bacteria.

Nitrogenase contains the two proteins molybdoferredoxin and azoferredoxin.

1. Azoferredoxin = component II, Fe protein, or nitrogenase reductase (a homodimer with each subunit between 24 and 36 kD). It is encoded by *Nif H* gene.
2. Molybdoferredoxin = component I, MoFe protein, or "nitrogenase" (a heterodimer of homodimers with total MW at about 220 kD). It is encoded by *Nif K* and *Nif D gene*.

Oxygen sensitivity of the Nitrogenase Complex

The nitrogenase enzyme complex is highly sensitive to oxygen. It is inactivated when exposed to oxygen, because O_2 reacts with the iron component of the proteins. Although this is not a problem for anaerobic bacteria, it could be a major problem for the aerobic species such as cyanobacteria (which generate oxygen during photosynthesis) and the free-living aerobic bacteria of soils, such as *Azotobacter* and *Beijerinckia*. These organisms have various methods to overcome the problem. For example:

- *Azotobacter* species have the highest known rate of respiratory metabolism of any organism, so they might protect the enzyme by maintaining a very low level of oxygen in their cells.
- *Azotobacter* species also produce large amounts of extracellular polysaccharide or exopolysaccharides. By maintaining water within the polysaccharide slime layer, these bacteria can limit the diffusion rate of oxygen to the cells.
- In the symbiotic nitrogen-fixing organisms such as *Rhizobium*, the root nodules can contain oxygen-scavenging molecules such as leghaemoglobin, which shows as a pink colour when the active nitrogen-

fixing nodules of legume roots are cut open. Leghaemoglobin may regulate the supply of oxygen to the nodule tissues in the same way as haemoglobin regulates the supply of oxygen to mammalian tissues.

- Some of the cyanobacteria have yet another mechanism for protecting nitrogenase: nitrogen fixation occurs in special cells (heterocysts) which possess only photosystem I (used to generate ATP by light-mediated reactions) whereas the other cells have both photosystem I and photosystem II (which generates oxygen when light energy is used to split water to supply H_2 for synthesis of organic compounds).
- The cyanobacteria *Gloeotheca* carries out photosynthesis during day and Nitrogen Fixation during night.

The genes encoding various components of nitrogen fixation system

NifJ = pyruvate flavodoxin reductase

nifF = flavodoxin

nifH = azoferredoxin or nitrogenase reductase or Fe protein

nifM = processing of NifH protein

nifK,D = molybdoferredoxin or MoFe protein, or "nitrogenase"

nifB,N,E,V,W,Z = MoFe cofactor synthesis

nifY = MoFe cofactor insertion

nifQ = molybdenum uptake

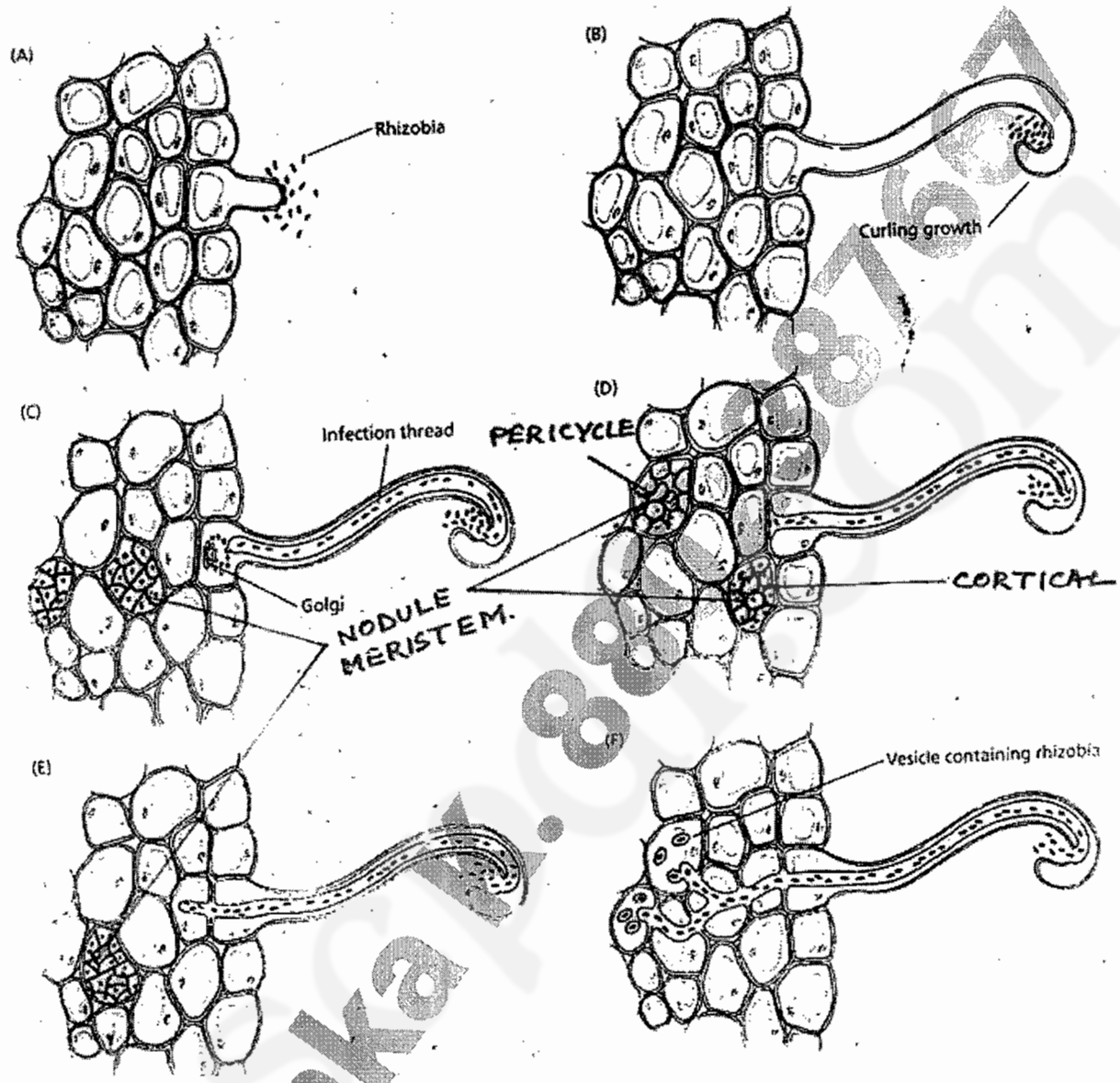
nifA,L,R = regulation

nifU,S = metal centre biosynthesis

nifX,T = function unknown (not necessary, at least under normal conditions)

The Establishment of Symbiosis for Nitrogen Fixation

The process is best understood in case of *Rhizobium* – Legume symbiosis and can be summarized through the following diagram.



The process involves a sequence of multiple interactions between the bacteria and the host roots. The events have four principal stages:

1. Multiplication of the rhizobia, colonization of the rhizosphere, and attachment to epidermal and root hair cells.
2. Characteristic curling of the root hairs and invasion of the bacteria to form an infection thread.
3. Nodule initiation and development in the root cortex. This stage is concurrent with stage 1.
4. Release of the bacteria from the infection thread and their differentiation as specialized nitrogen fixing cells.

Stage I

Rhizobia are free-living, saprophytic soil bacteria. Their numbers in the soil are highly variable. In the presence of host roots, the bacteria are encouraged to multiply and colonize the rhizosphere. The initial attraction of rhizobia to host roots involves positive chemotaxis due to a variety of amino acids, sugars, and organic acids released by roots. In some cases, the attraction of rhizobia is due to flavonoids such as Luteolin, Diadzein and Naringenin.

Once rhizobia have colonized the rhizosphere, they begin to synthesize morphogenic signal molecules called nodulation factors, or nod factors. These factors are lipo-chitooligosaccharides.

They induce several significant changes in the growth and metabolism of the host roots – such as increased root hair production and the development of shorter, thicker roots.

Stage II

As a result of nod factor signaling, the root hairs also develop branching and curl at the tip. After this, the bacteria aggregated on the root surface can initiate infection.

Rhizobia-host specificity is genetically determined. At surface level, recognition involves two classes of molecules: lectins and complex polysaccharides. Lectins are small, nonenzymatic proteins synthesized by the host and have the particular ability to recognize and bind to specific complex carbohydrates. Individual legume species each produce different lectins with different sugar-binding specificities. Lectins of hosts recognize complex polysaccharides found on the surface of the potential symbiont.

Experiments have also indicated the involvement of a calcium-binding protein, called rhicadhesin, located on the surface of the rhizobial cell. Rhicadhesin is required for attachment in addition to lectin.

After attachment, the bacterium penetrates the host cell wall and enters the space between the wall and the plasma membrane. The process includes some degree of wall degradation as Rhizobia release enzymes such as pectinase, hemicellulase and cellulase.

Once the rhizobia reach the outer surface of the plasma membrane, tip growth of the root hair ceases and the cell membrane begins to invaginate. The result is a tubular intrusion into the cell called an infection thread, which contains the invading rhizobia.

The infection thread elongates inwards. The infection thread continues to elongate until it reaches the base of the root hair cell. Here, it again breaches the cell wall for the bacteria to gain access to the next cell in their path.

The infection process continues into successive cells in the cortex. As the infection thread moves through the root hair into the cortex, the bacteria continue to multiply. When the thread reaches the developing nodule, it branches so that many individual cells in the young nodule become infected.

Stage III

Rhizobia also release mitogenic signals that stimulate localized cell divisions in the root cortex. These cell divisions form the primary nodule meristem. This is the region in which the nodule will develop. A second centre of cell division arises in the pericycle. Eventually these two masses of dividing cells will fuse to form the complete nodule.

The nature of the mitogenic signal is unknown, although there is some evidence that the plant hormones Cytokinin and Ethylene might be involved.

Stage IV

When the infection thread reaches a cell deep in the cortex, it bursts and the bacteria are engulfed by endocytosis into endosomes.

At this time the cell goes through several rounds of mitosis – without cytokinesis – so the cell becomes polyploid.

The bacteria cease dividing, enlarge and differentiate into specialized nitrogen-fixing cells called bacteroids. The bacteroids remain surrounded by a membrane, now called the peribacteroid membrane.

Differentiation into a bacteroid is involves the synthesis of the enzymes and other factors that the organism requires for the principal task of nitrogen fixation.

As the nodule increases in size due to the activity of the nodule meristem, bacteria continue to invade the new cells. Vascular connections are established with the main vascular system of the root. These vascular connections serve to import photosynthetic carbon into the nodule and export fixed nitrogen from the nodule to the plant.

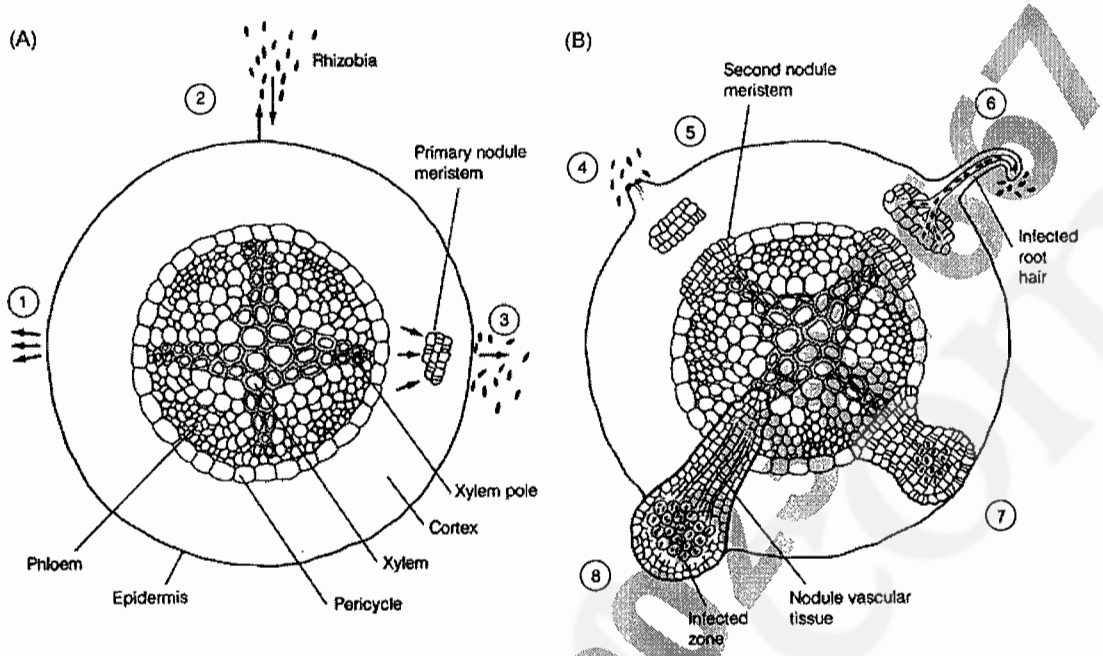
Legumes of temperate origin like *Pisum*, *Vicia* and *Trifolia* export ammonia (fixed nitrogen) as Amide through xylary translocation. On the other hand, the legumes of tropical origin like *Glycine*, *Phaseolus* and *Arachis* export ammonia (fixed nitrogen) as Ureids through xylary translocation. Common ureids include Allantoin and Citrullic Acid.

A short summary of symbiotic events

Stages in the initiation and development of a soybean root nodule is shown in the next figure.

Figure (A) Events involved in the initiation of the nodule: (1) the root excretes substances; (2) these substances attract rhizobia and stimulate them to produce cell-division factors; (3) cells in the root cortex divide to form the primary nodule meristem.

Figure (B) Stages of infection and nodule formation: (4) bacteria attach to the root hair; (5) cells in the pericycle near the xylem poles are stimulated to divide; (6) the infection thread forms and extends inward as the primary nodule meristem and the pericylce continue to divide; (7) the two masses of dividing cells fuse into a single clump while the infection thread continues to grow; (8) the nodule elongates and differentiates, including the vascular connection to the root stele. Bacteroids are released into the cells in the centre.



Benefits of symbiotic N_2 fixation

In croplands, nitrogen is often the limiting factor for growth and biomass production in all environments where there is suitable climate and availability of water to support life. Typically a hectare of *legume-Rhizobium* association will fix 25 to 60 kg of dinitrogen annually, while nonsymbiotic organisms fix less than 5 kg ha^{-1}. In term of biomass increment, field studies suggest a gain of at least 20%, that too without contaminating the soil with chemical fertilizers.

The Genetics of Nitrogen Fixation

1. In nitrogen fixation, the nitrogenase complex takes up a key position. The encoding and the regulation of this protein is controlled by a certain DNA region, the *nif*-region, that contains 16 (or 17) genes in the case of *Klebsiella*. The *nif*-genes belong to seven different operons (transcription units). Except for one gene that is located on the complementary strand, all of them are located on the same (the 'encoding') strand. Please refer to the preceding list for a detailed description of *nif* operons.
2. *fix* genes have also been reported from symbiotic diazotrophs. *fix-X* gene encodes ferredoxin, which is the electron carrier in nitrogen fixation.
3. The symbiosis related genes are located on the *Sym* plasmid of *Rhizobium*.
4. Phenolic compounds secreted by the roots, e.g. luteolin, serve as signals. They activate the *nodD* gene of the nodule bacterium that is the master gene for symbiosis and regulates further genes of the '*nod*-box': *nodA, nodB, and nodC*. These genes occur in all nodule bacteria (common *nod*-genes), and a further group of nod-genes (*nodH...*) participates in the reaction between bacterium and host plant. The production of an additional signal molecule is followed by the first visible morphological change: the root hairs start to bend.
5. *E-Nod* genes in the hosts participate in the reaction between bacterium and host plant.
6. Bacteria of the genus *Bradyrhizobium* have a two-component system: *nodV* and *nodW*. Here, too, both components seem to be necessary for nodulation.

7. Besides the *nod*-genes, a further group of genes, the *hsn* genes (host specificity of nodulation) are known.

Integration of Ammonium into Organic Molecules to Generate Amino Acids

Plants have 4 principal pathways, as depicted below, for integration of ammonium into organic molecules to generate Amino Acids. Asparagine is the most preferred amino acid for storage because it gives best C:N ratio, i.e. 4:2, which is better than any amino acid.

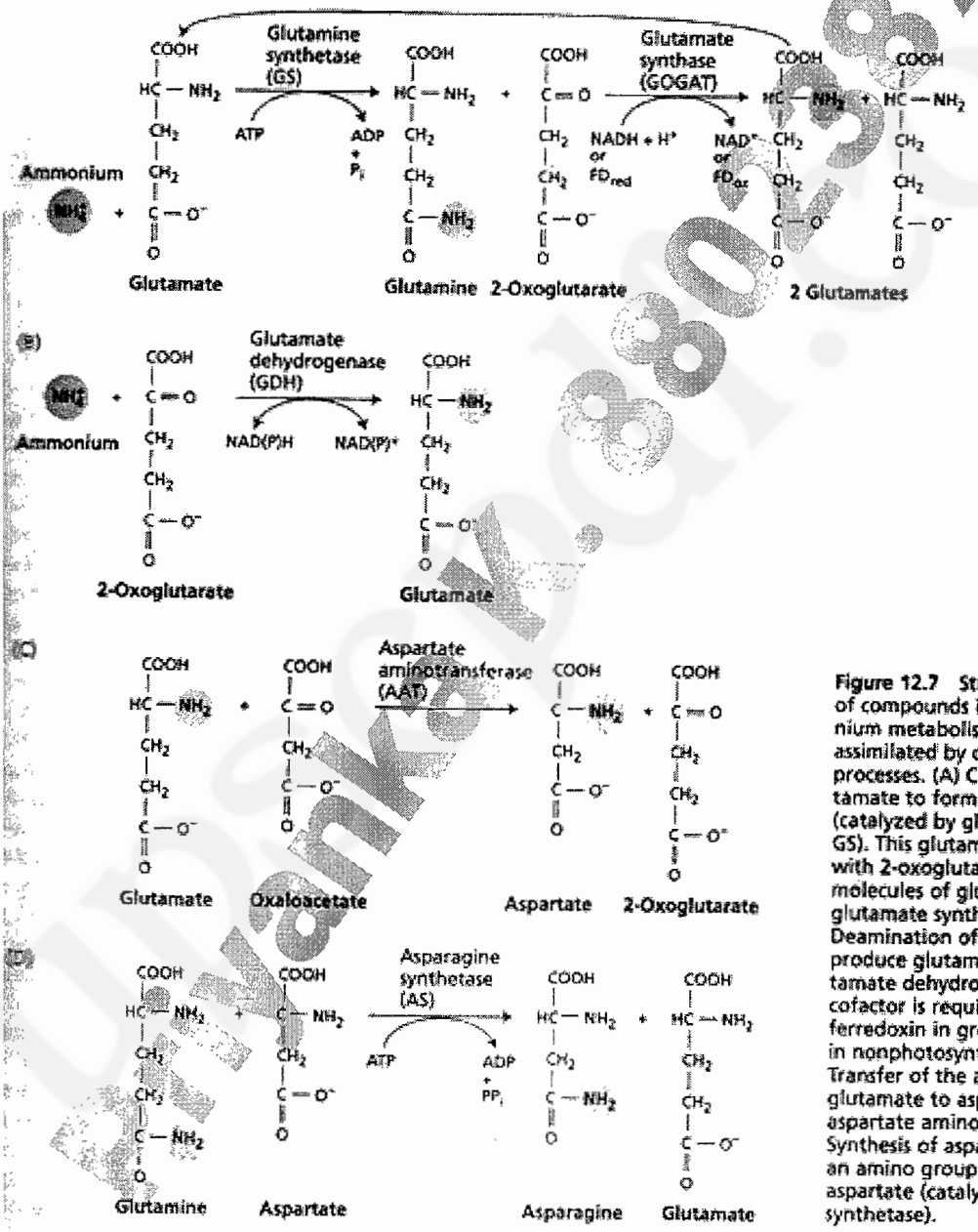


Figure 12.7 Structure and pathways of compounds involved in ammonium metabolism. Ammonium can be assimilated by one of several processes. (A) Combination with glutamate to form the amide glutamine (catalyzed by glutamine synthetase, GS). This glutamine then combines with 2-oxoglutarate to form two molecules of glutamate (catalyzed by glutamate synthase, GOGAT). (B) Deamination of 2-oxoglutarate to produce glutamate (catalyzed by glutamate dehydrogenase). A reduced cofactor is required for the reaction: ferredoxin in green leaves and NADH in nonphotosynthetic tissue. (C) Transfer of the amino group from glutamate to aspartate (catalyzed by aspartate aminotransferase). (D) Synthesis of asparagine by transfer of an amino group from glutamine to aspartate (catalyzed by asparagine synthetase).

Enzymes and Co-enzymes

Introduction to enzymes

Enzymes are bio-organic polymers which act as the catalysts in biological processes. The enzyme catalyzed processes in biological systems include:

- Nearly all the biochemical transformations in the cell;
- A large number of biomechanical functions, such as unwinding the DNA double helix; and
- Most of the bio-energetic transformations.

Chemically, **two classes of biopolymers are now known to be enzymes**.

1. **Proteins** – *Most of the known enzymes are proteins; either with single peptide or multi-peptide construction.* Till the mid-1980s, it was believed that all the enzymes are proteins. However, the discovery of RNA enzymatic activity changed this notion.
2. **RNA** – The catalytic activity of RNA was discovered by Thomas Chech in mid-1980s as the self-splicing (autocatalytic) Group-I intron in the ciliated protozoan *Tetrahymena thermophila*. Around the same time Sidney Altman discovered Ribonuclease-P that processes pre-tRNA. Both self-splicing (autocatalytic) Group-I intron and Ribonuclease-P are RNA molecules showing catalytic activity. Such RNA molecules are called **Ribozymes**. Currently about 29 ribozymes are well characterized, which includes the Peptidyl Transferase enzyme of the Ribosomes.

In experimental systems, ssDNA molecules have also shown catalytic activities and they have been termed DNAzymes. However, no such catalytic DNA molecule has been found *in vivo*.

Explanatory Note: A **catalyst** is a substance that, when added to a system, speeds up a process without altering the energetics or the equilibrium state of the process. In other words, a catalyst operates in a thermodynamically permissible situation, where it serves to accelerate the attainment of the process equilibrium. Catalysts show three essential properties:

- They do not get consumed in or modified by the process
- They act in very small quantities
- They do not alter the energetics or equilibrium state relation of the process.

Characteristics of Enzymes

The enzymes are remarkable molecular devices that determine the patterns of chemical or energetic transformations in biological systems. They **show the characteristics of both ordinary chemical catalysts and bio-organic polymers.**

Characteristic feature of enzymes like ordinary chemical catalysts

1. Enzymes accelerate reactions by factors of as much as a million or more. The greatest rate acceleration (i.e. 10^{17} times) is brought about by the enzyme Orotidine monophosphate (OMP) Decaroxylase.

2. Enzymes act by bringing down the activation energy of the transformation.
3. Like any other catalyst, the enzymes are required in very small amounts.
4. The enzymes remain unaffected even after the process ends.
5. The enzymes do not change the energetic relations of the process. In other words, the enzymes can not make a thermodynamically unfavourable process possible; they merely make a thermodynamically feasible process faster.
6. The enzymes do not alter the equilibrium of the reaction. Like ordinary catalysts, the enzymes also accelerate the reverse reaction by the same factor.

Characteristic feature of enzymes like bio-organic polymers

1. Crystallization and chemical analysis of any known enzyme would establish that they are either protein or RNA.
2. Like any other protein, most of the enzymes (except ribozymes) show amphoteric nature.
3. Like any other protein, most of the enzymes (except ribozymes) show protease sensitivity.
4. Enzymes show a characteristic three dimensional structure, without which there can be no catalytic action. Catalysis takes place at a particular site on the enzyme called the *active site*. The active site has a specific three dimensional structure.
5. Enzymes are highly specific both in the reactions that they catalyze and in their choice of *substrates. An enzyme usually catalyzes a single chemical reaction or a set of closely related reactions.* They can also distinguish between D and L isomers of the same molecule.*Side reactions leading to the wasteful formation of by-products are rare in enzyme-catalyzed reactions,* in contrast with uncatalyzed ones. *The specificity of an enzyme is due to the precise interaction of the substrate with the enzyme. This precision is a result of the intricate three-dimensional structure of the enzyme protein.*
6. Enzyme action proceeds under moderate reaction conditions, which includes body temperature, atmospheric pressure and near neutral pH in most cases.
7. Like other bio-organic polymers, the enzymes are also susceptible to damage or inactivation by too high or too low temperature, pH or ionic strength.
8. A number of chemical substances can inhibit enzyme action by binding to them in an undesirable way. This kind of inhibition is normally not seen in case of ordinary chemical catalysts.
9. In many reactions, *the energy of the reactants is converted with high efficiency into a different form.* For example, in photosynthesis, light energy is converted into chemical-bond energy through an ion gradient. In mitochondria, the free energy derived from food is converted ultimately into the free energy of adenosine triphosphate [ATP]. Other enzymes may then use the chemical-bond energy of ATP in many ways. *The enzyme myosin converts the energy of ATP into the mechanical energy of contracting muscles*. The mechanisms of these energy-transducing processes are absent in ordinary chemical catalysts.
10. Enzymatic catalysis is regulated by a number of ordinary cellular mechanisms, which also serve to regulate the function of other cellular proteins or other macro-molecules.

Types of Enzymes

Enzymes are now classified based on the types of reactions that they catalyze. The commonly used names for most enzymes describe the type of reaction catalyzed, followed by the suffix *-ase*. For example, dehydrogen*ases* remove hydrogen atoms, prote*ases* hydrolyze proteins, and isomer*ases* catalyze rearrangements in configuration. Modifiers may precede the name to indicate the substrate (*xanthine* oxidase), the source of the enzyme (*pancreatic* ribonuclease), its regulation (*hormone-sensitive* lipase), or a feature of its mechanism of action (*cysteine* protease). Where needed, alphanumeric designators are added to identify multiple forms of an enzyme (eg, RNA polymerase *III*; protein kinase Cβ).

To address ambiguities and bring some consistency to the classification of enzymes, *in 1964 the International Union of Biochemistry* (IUB) established an *Enzyme Commission* to develop a nomenclature for enzymes. According to the codes provided by the enzyme commission,

Reactions are divided into six major groups numbered 1 through 6. Accordingly there are **6 major classes of enzymes**, each one catalyzing a specific type of reaction:

Enzyme Class 1. **Oxidoreductases –** carries out Transfer of electrons; for example: Lactate dehydrogenase.

Enzyme Class 2. **Transferases –** carries out Group transfer across molecules; for example: Nucleoside monophosphate kinase (NMP kinase).

Enzyme Class 3. **Hydrolases –** carries out Hydrolysis reactions (transfer of functional groups to water); for example: Chymotrypsin.

Enzyme Class 4. **Lyases –** carries out Addition or removal of groups to form double bonds; for example: Fumarase

Enzyme Class 5. **Isomerases –** carries out Isomerization (*intramolecular* group transfer); for example: Triose phosphate isomerase.

Enzyme Class 6. **Ligases –** carries out Ligation of two substrates at the expense of ATP hydrolysis; for example: Aminoacyl-tRNA synthetase

The reaction groups are subdivided and further subdivided, so that a *four-digit number preceded by the letters EC* for Enzyme Commission could precisely identify all enzymes. For example, the enzyme Peptidyl –L– amino acid hydrolase is denoted by EC 3.4.17.1

In the systematic name of an enzyme:

- EC stands for Enzyme Commission
- First digit indicates the enzyme's major class – based on the reaction type it catalyses.
- The second number denotes the sub-class of an enzyme – based on the kind of bond / substrate the enzyme acts on.
- The third number denotes sub-subclass – based on the reaction mechanisms or specifics.
- The fourth number is arbitrarily assigned as the serial number to the enzyme within a particular sub-subclass under a particular class of enzyme.

An illustration: The IUB name of hexokinase is ATP:D-hexose-6-phosphotransferase or E.C. 2.7.1.1. This name identifies hexokinase as a member of class 2 (transferases), subclass 7 (transfer of a phosphoryl group), sub-subclass 1 (alcohol is the phosphoryl acceptor), the last 1 means that it is the first enzyme to be enlisted in this sub-subclass. The designation "hexose-6" indicates that the alcohol phosphorylated is on carbon six of a hexose.

Enzyme structure

Most Enzymes are proteins and the remaining are Ribonucleic Acid (RNA). So far, there is no third group of biomolecules that has shown catalytic abilities *in vivo*.

The biological catalytic role played by the enzymes is primarily dependent on the enzyme-substrate interaction due to which the activation energy for a chemical transformation is lowered and reaction rate is accelerated. The interaction of the enzyme with the substrate depends crucially on the three-dimensional structure of the enzyme. The factors like extremes of temperature, pH, ionic strength etc. which cause structural deformities in the enzymes often lead to the loss of catalytic activity of enzymes.

Similarly, most of the enzyme inhibitors and regulators act by influencing the enzyme structure.

There can be no doubt that structure is the most important foundation beneath almost every facet of enzyme action.

The structural attributes of proteinaceous enzymes and ribozymes are discussed separately below.

The structural attributes of proteinaceous enzymes

Like all proteins, enzymes are made as long, linear chains of amino acids that fold to produce a three-dimensional product. There are four orders of protein structure: **primary structure,** the sequence of the amino acids in a polypeptide chain; **secondary structure,** the folding of short (3- to 30-residue), contiguous segments of polypeptide into geometrically ordered units; **tertiary structure,** the assembly of secondary structural units into larger functional units such as the mature polypeptide and its component domains; and **quaternary structure,** the number and types of polypeptide units of oligomeric proteins and their spatial arrangement.

All the proteinaceous enzymes show the first three levels of protein structure but only the oligomeric enzymes display the fourth order of protein structure.

The proteinaceous enzymes range from just 62 amino acid residues in size for the monomer of 4-oxalocrotonate tautomerase, to over 2,500 residues in the animal fatty acid synthase. **Most enzymes are made up of more than 100 amino acid residues, which gives them a mass greater than 10 kd and a diameter of more than 25 Å.**

The activities of enzymes are determined by their three-dimensional (3-D)structure, which is an outcome of the secondary and tertiary folding in most of the cases (in oligomeric enzymes, even the quarternary structure is an important determinant of the 3-D structure).

The 3D structure attained after protein synthesis and folding is called the native structure. The 3-D structure in which the enzyme shows its catalytic activity is known as the active structure. For many enzymes, the native structure and the active structure are one and same. However, for many other enzymes this is not the case. These enzymes need to be converted from their native structure into the active structure by one of the following factors:

1. Close range interaction with the substrate
2. Binding of a co-enzyme
3. Binding of a metallic co-factor
4. Optimal pH (as in case of lysosomal enzymes)
5. Binding of an activator
6. Binding of an allosteric modulator, *etc.*

Most enzymes are much larger than the substrates they act on, and only a very small portion of the enzyme (around 3–4 amino acids) is directly involved in catalysis. The region that contains these catalytic residues, binds the substrate, and then carries out the reaction is known as the active site. Enzymes can also **contain sites that bind** cofactors, which are needed for catalysis. Some enzymes also have binding sites for small molecules, which often serve regulatory purposes. These sites are called **regulatory sites**. The binding of regulators can serve to increase or decrease the enzyme's activity, providing a means for enzyme regulation.

Most of the amino acid residues in an enzyme are not in contact with the substrate. The "extra" amino acids serve as a scaffold to create the three-dimensional active site from amino acids that are far apart in the primary structure.

The part of the enzyme that is directly involved in catalysis is the active site. It is the region that contains the catalytic amino acid residues, binds the substrate, and then carries out the reaction.

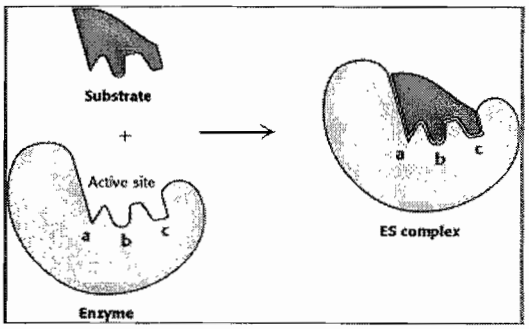
The Active Sites of Enzymes Have Some Common Features, which include:

The active site of an enzyme is the region that binds the substrates (and the cofactor, if any). It also contains the residues that directly participate in the making and breaking of bonds. These residues are called the *catalytic groups*. In essence, *the interaction of the enzyme and substrate at the active site promotes the formation of the transition state*. The active site is the region of the enzyme that most directly lowers the $\Delta G^{\ddagger}$ of the reaction, which results in the rate enhancement characteristic of enzyme action. Although enzymes differ widely in structure, specificity, and mode of catalysis, a number of generalizations concerning their active sites can be stated:

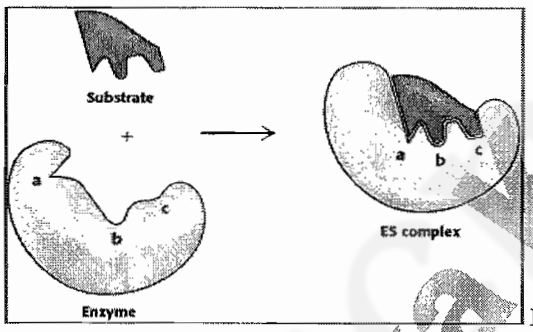
1. *The active site is a three-dimensional cleft formed by groups that come from different parts of the amino acid sequence.* In lysozyme, an enzyme that degrades the cell walls of some bacteria, the important groups in the active site are contributed by residues numbered 35, 52, 62, 63, 101, and 108 in the sequence of the 129 amino acids.
2. *The active site takes up a relatively small part of the total volume of an enzyme.*
3. *Active sites are clefts or crevices.* In all enzymes of known structure, substrate molecules are bound to a cleft or crevice.
4. *Water is usually excluded from the active site unless it is a reactant.* The nonpolar character of much of the cleft enhances the binding of substrate as well as catalysis.
5. *Substrates are bound to enzymes by multiple weak attractions.* ES complexes usually have equilibrium constants that range from 10^{-2} to 10^{-8} M, corresponding to free energies of interaction ranging from about -3 to -12 kcal mol^{-1} (from -13 to -50 kJ mol^{-1}). The noncovalent interactions in ES complexes are much weaker than covalent bonds, which have energies between -50 and -110 kcal mol^{-1} (between -210 and -460

kJ mol^{-1}). Electrostatic interactions, hydrogen bonds, van der Waals forces, and hydrophobic interactions mediate reversible interactions of biomolecules.

6. *The specificity of binding depends on the precisely defined arrangement of atoms in an active site.* Because the enzyme and the substrate interact by means of short-range forces that require close contact, a substrate must have a matching shape to fit into the site. Emil Fischer's analogy of the lock and key, expressed in 1890, has proved to be highly stimulating and fruitful. However, we now know that enzymes are flexible and that the shapes of the active sites can be markedly modified by the binding of substrate, as was postulated by Daniel E. Koshland, Jr., in 1958. The active sites of some enzymes assume a shape that is complementary to that of the transition state only *after* the substrate is bound. This process of dynamic recognition is called *induced fit*.



Lock-and-Key Model



Induced Fit Model

The specificity of an enzyme is due to the precise interaction of the substrate with the enzyme. This precision is a result of the intricate three-dimensional structure of the enzyme protein. The binding of enzyme to its substrate is based on structural complementarity.

The interaction of the enzyme and substrate at the active site promotes the formation of the transition state. Although enzymes differ widely in structure, specificity, and mode of catalysis, yet the active site after binding to the substrate lowers the $\Delta G^{\ddagger}$ of the reaction, which results in the rate enhancement characteristic of enzyme action.

The structural attributes of ribozymes

A **ribozyme** (**ribo**nucleic acid en**zyme**, also called RNA enzyme or catalytic RNA) is an RNA molecule that catalyzes a chemical reaction. Many natural ribozymes catalyze either their own cleavage or the cleavage of other RNAs, but they have also been found to catalyze the aminotransferase activity of the ribosome.

The ribozymes are the polymers of ribonucleotides. In most of the cases, the ribozymes have a single ribonucleic acid chain, but this chain folds in many possible ways to give rise to catalytic activity. After the

final folding, even in ribozymes an active site develops. There is usually not any regulatory site available in a ribozymes. Some important examples of ribozymes with their structural schematics is shown below.

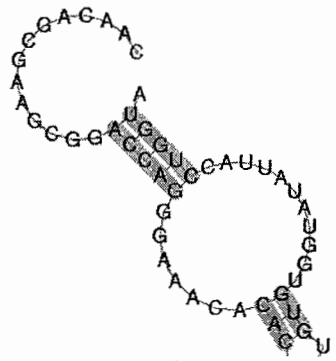


Figure: Hairpin Ribozyme: Found in RNA satellites of plant viruses.

Figure: Hammerhead Ribozyme: Found in several of the **viroids** and **satellite RNAs** associated with plant RNA viruses and other species

Most of the ribozymes are autocatalytic. However, those which are not autocatalytic normally interact with the substrate in Induced Fit manner. Like protein enzymes, even a ribozyme lowers the $\Delta G^{\ddagger}$ of the reaction, which results in the rate enhancement characteristic of enzyme action.

Mechanism of enzyme action

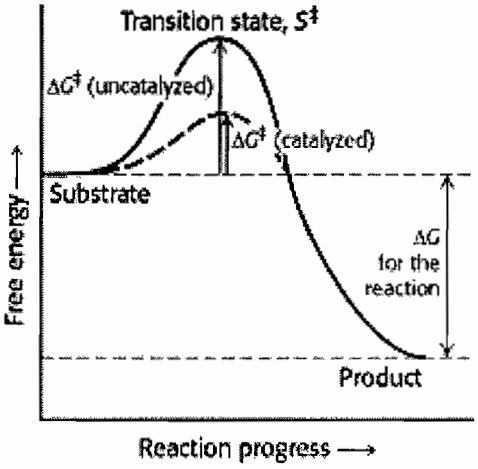
Enzymes serve to accelerate biochemical reactions by factors of as much as a million or more. The greatest rate acceleration (i.e. 10^{17} times) is brought about by the enzyme Orotidine monophosphate (OMP) Decaroxylase. *The enzymes do so by bringing down the activation energy* of the transformation. However, *the enzymes do not change the energetic relations of the process*. In other words, the enzymes can not make a thermodynamically unfavourable process possible; they merely make a thermodynamically feasible process faster. And finally, the *enzymes do not alter the equilibrium of the reaction*. Like ordinary catalysts, the enzymes also accelerate the reverse reaction by the same factor.

A description of enzyme function in terms of thermodynamic relations

*Two **unrelated** thermodynamic properties of the reaction are important*:

The free-energy difference (ΔG) between the products and reactants: It determines whether the reaction will be spontaneous or will need energy input. The following considerations are important about reaction energetics.

A reaction can occur spontaneously only if ΔG is negative. Such reactions are said to be exergonic.



A system is at equilibrium and no net change can take place if

ΔG is zero.

A reaction cannot occur spontaneously if ΔG is positive. An input of free energy is required to drive such a reaction. These reactions are termed endergonic.

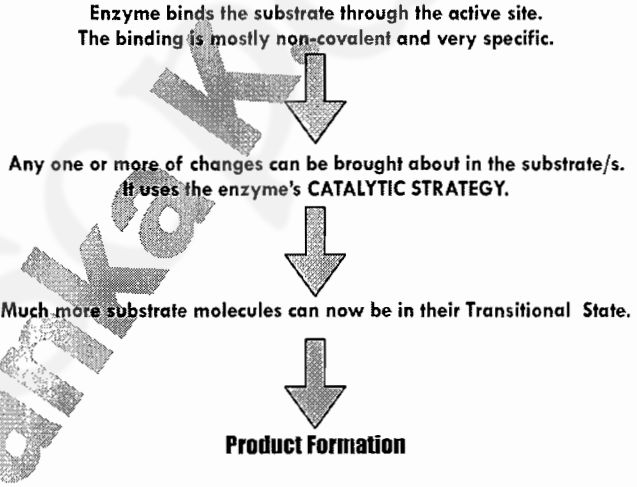
The ΔG of a reaction depends only on the free energy of the products (the final state) minus the free energy of the reactants (the initial state). *The ΔG of a reaction is independent of the path (or molecular mechanism) of the transformation.* The mechanism of a reaction has no effect on ΔG. *The ΔG provides no information about the rate of a reaction.* A negative ΔG indicates that a reaction *can* occur spontaneously, but it does not signify whether it will proceed at what rate.

The energy required to initiate the conversion of reactants to products or Activation Energy. It determines the rate of the reaction. *Enzymes affect only the activation energy and not ΔG.* Hence the *enzymes control only the rate of the reaction.* The rate of a reaction depends on the *free energy of activation* ($\Delta G^{\ddagger}$), which is largely unrelated to the ΔG of the reaction. A chemical reaction of substrate S to form product P goes through a *transition state* $S^{\ddagger}$ that has a higher free energy than does either S or P.

The transition state is the most seldom occupied state along the reaction since it is the one with the highest free energy. The difference in free energy between the transition state and the substrate is called the *Gibbs free energy of activation* or simply the *activation energy*, symbolized by $\Delta G^{\ddagger}$.

The energy of activation, or $\Delta G^{\ddagger}$, does not enter into the final ΔG calculation for the reaction, because the energy input required to reach the transition state is returned when the transition state forms the product.

It is obvious from the above diagram that enzymes function to lower the activation energy, or, in other words, *enzymes facilitate the formation of and stabilize the the transition state.*



Binding between the Enzyme and the Substrate

In any enzyme's three dimensional structure, there is a specific site where it binds to the substrate for catalysis. This site is called the **Active Site.** The structure and chemical properties of the active site allow the recognition and binding of the substrate.

The active site is usually a small pocket at the surface of the enzyme that contains residues responsible for the susbtrate specificity (charge, hydrophobicity, steric hindrance) and catalytic residues which often act as proton donors or acceptors or are responsible for binding a cofactor such as Pyridoxal, Thiamine or NAD.

By utilizing the various types of intermolecular forces – mostly **non-covalent, cognate type** – *the active site brings substrates together in an optimal orientation*, and facilitate making and breaking chemical bonds. They catalyze reactions *by stabilizing transition states,* the highest-energy species in reaction pathways.

The **concept of Binding Energy:** The *binding energy* is the free energy released in the formation of a large number of weak interactions between the enzyme and the substrate. This binding energy serves two purposes:

1. It establishes substrate specificity
2. Increases catalytic efficiency.

Only the correct substrate can participate in most or all of the interactions with the enzyme and thus maximize binding energy, accounting for the substrate specificity exhibited by most enzymes.

Interactions between the enzyme and the substrate not only favor substrate binding but stabilize the transition state, thereby lowering the activation energy. The binding energy can also promote structural changes in both the enzyme and the substrate that facilitate catalysis.

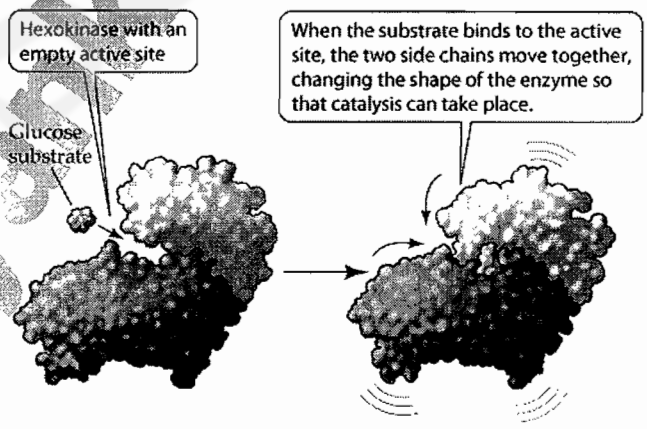
There are two dominant models of how enzymes bind to the substrate:

1. the lock-and-key model
2. the induced fit model.

The **Lock and Key model** (E. Fischer, 1894) states that the active sites and their respective substrates have specified pre-formed structures which are complementary to each other. Hence, the substrate fits into the enzyme's active site, and the reaction occurs. This model is shown below.

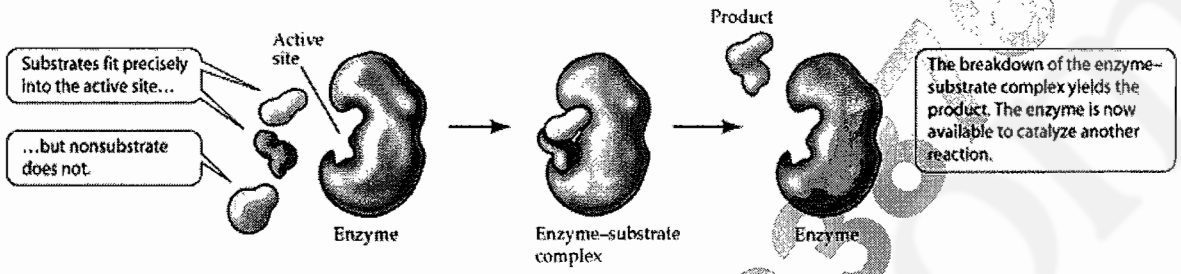
The **induced fit model** was submitted by Koshland in 1959, based on his work with X-ray diffraction analysis. He suggested that the active site of an enzyme was more flexible than previously thought, and that it moulded to the shape of the substrate to interact with it. The binding energy released during enzyme substrate interaction can also promote structural changes in both the enzyme and the substrate that facilitate catalysis.

One example of induced fit with reference to the enzyme hexokinase is shown below.



Catalytic Strategies

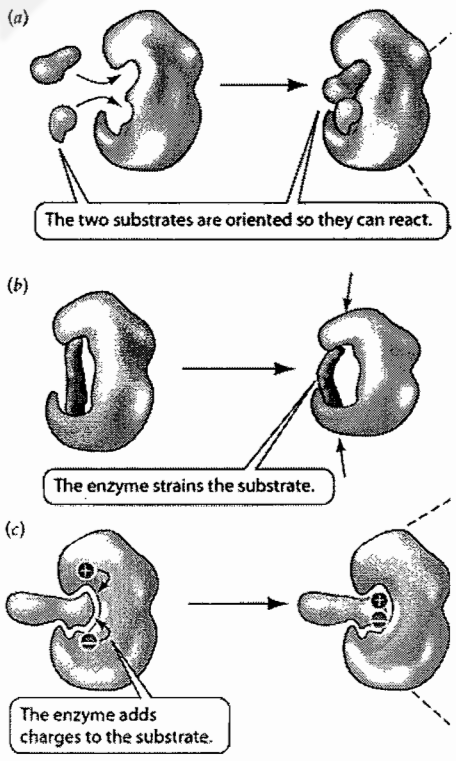
The **active site** generally takes the form of a cleft or pocket, often located at the interface between enzyme subunits and recruit amino acyl residues drawn from diverse portions of the polypeptide chain and also from more than one enzyme subunit. The three-dimensional active site shields substrates from solvent and facilitates catalysis. The active site also binds and orients cofactors or prosthetic groups.



Enzymes use various combinations of **three general mechanisms** to achieve dramatic catalytic enhancement of the reaction rates.

1. **Catalysis by Proximity**: For molecules to react, they must come within bond-forming distance of one another. When an enzyme binds substrate molecules at its active site, it creates a region of high local substrate concentration. This environment also orients the substrate molecules spatially in a position ideal for them to interact, resulting in rate enhancements of at least a thousand fold.

2. **Catalysis by Strain**: Enzymes that catalyze lytic reactions that involve breaking a covalent bond typically bind their substrates in a conformation slightly unfavorable for the bond that will undergo cleavage. The resulting strain stretches or distorts the targeted bond, weakening it and making it more vulnerable to cleavage.



3. **Catalysis by causing change in the substrate:** It has several mechanisms.

   3a. **Acid-Base Catalysis:** The ionizable functional groups of aminoacyl side chains and those of prosthetic groups (where present) contribute to catalysis by acting as acids or bases. Acid-base catalysis can be either specific or general. By "specific" we mean only protons (H^+) or OH^- ions. In **specific acid** or **specific base catalysis,** the rate of reaction is sensitive to changes in the concentration of protons but independent of the concentrations of other acids (proton donors) or bases (proton acceptors) present in solution or at the active site. Reactions whose rates are responsive to *all* the acids or bases present are said to be subject to **general acid** or **general base catalysis.**

   3b. **Covalent Catalysis:** The process of **covalent catalysis** involves the formation of a covalent bond between the enzyme and one or more substrates. The modified enzyme then becomes a reactant.

Covalent catalysis introduces a new reaction pathway whose activation energy is lower—and therefore is faster. The chemical modification of the enzyme is, however, transient. On completion of the reaction, the enzyme returns to its original unmodified state. Its role thus remains catalytic. Covalent catalysis is particularly common among enzymes that catalyze group transfer reactions. Residues on the enzyme that participate in covalent catalysis generally are cysteine or serine and occasionally histidine.

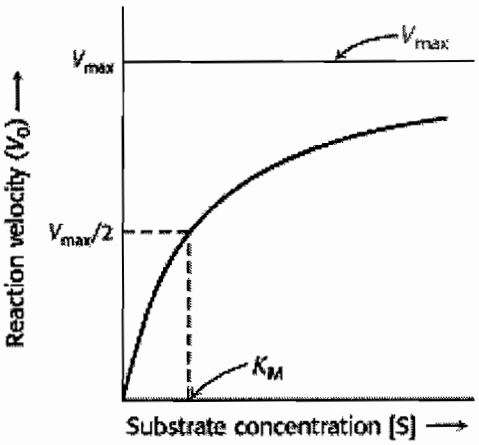
3c. **Metal ion catalysis**. Metal ions can function catalytically in several ways. For instance, a metal ion may serve as an electrophilic catalyst, stabilizing a negative charge on a reaction intermediate. Alternatively, the metal ion may generate a nucleophile by increasing the acidity of a nearby molecule, such as water in the hydration of CO_2 by carbonic anhydrase. Finally, the metal ion may bind to substrate, increasing the number of interactions with the enzyme and thus the binding energy. This strategy is used by NMP kinases.

3d. **Electrostatic catalysis**. Where the enzyme acts as an electrostatic stabilizing agent, mostly through some cofactor such as Mg++ ions, as seen in the plant enzyme RUBISCO.

3e. **Redox catalysis.** As seen the OEC enzyme associated with the Plant PS II, or in the bacterial enzyme Nitrogenase Complex, the enzyme cofactor [such as Mn ions, or Fe4-S4 cluster, respectively] acts as a strong redox agent, that brings about substrate transformation.

3f. **Combinatorial catalysis.** When the same enzyme employs a multitude of catalytic strategies – it is called combinatorial catalysis. One of the best studied examples is the Lysozyme – found in human secretions such as tears that breaks-down the bacterial cell wall, using several catalytic strategies together. This mechanism was first shown by Philips et al in 1950s.

The various catalytic strategies ensure that the formation of transition state substrates is maximized and also that the transition state substrates are stabilized. As an outcome, the rate of product formation is greatly increased.



The Relation between Substrate Concentration and Reaction Rates [The Michaelis – Menten Model]

For many enzymes, the rate of catalysis V_0, which is defined as the number of moles of product formed per second, varies with the substrate concentration [S] in a manner shown below.

The rate of catalysis rises linearly as substrate concentration increases and then begins to level off and approach a maximum at higher substrate concentrations.

In 1913, **Leonor Michaelis** and **Maud Menten** proposed a simple model to account for these kinetic characteristics. The critical feature in their treatment is that a specific ES complex is a necessary intermediate in catalysis. The model is the simplest one that accounts for the kinetic properties of many enzymes.

It proposed that:

An enzyme E combines with substrate S to form an ES complex, with a rate constant k_1. The ES complex has two possible fates. It can dissociate to E and S, with a rate constant k_{-1}, or it can proceed to form product P,

with a rate constant k_2. Again, we assume that almost none of the product reverts to the initial substrate, a condition that holds in the initial stage of a reaction before the concentration of product is appreciable.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

The rates of formation and breakdown of ES are given by:

$$\text{Rate of formation of ES} = k_1[E][S]$$

$$\text{Rate of breakdown of ES} = (k_{-1} + k_2)[ES]$$

To simplify the calculations, a new constant was defined, K_M, called the *Michaelis constant:*

$$K_M = \frac{k_{-1} + k_2}{k_1}$$

K_M has the units of concentration. K_M is an important characteristic of enzyme-substrate interactions and is independent of enzyme and substrate concentrations.

The Michaelis-Mented Equation is:

$$V_0 = V_{max} \frac{[S]}{[S] + K_M}$$

This equation accounts for the kinetic data given in the figure above. At very low substrate concentration, when [S] is much less than K_M, $V_0 = (V_{max}/K_M)[S]$; that is, the rate is directly proportional to the substrate concentration. At high substrate concentration, when [S] is much greater than K_M, $V_0 = V_{max}$; that is, the rate is maximal, independent of substrate concentration.

The meaning of K_M is evident from equation above. When [S] = K_M, then $V_0 = V_{max}/2$. Thus, K_M*is equal to the substrate concentration at which the reaction rate is half its maximal value.* K_M is an important characteristic of an enzyme-catalyzed reaction and is significant for its biological function.

A low K_M means high substrate affinity of the enzyme, while a high K_M means a low substrate affinity of the enzyme.

For most enzymes, K_M lies between 10^{-1} and 10^{-7} M. The K_M value for an enzyme depends on the particular substrate and on environmental conditions such as pH, temperature, and ionic strength.

Measurement the catalytic action of enzymes

The Katal (symbol: kat) is the SI unit of catalytic activity. It is that amount of a catalyst that catalyzes a reaction rate of 1 mole of substrate per second.

The enzyme unit (U) is a non-SI unit for the amount of a particular enzyme. One U is defined as the amount of the enzyme that catalyzes the conversion of 1 micro mole of substrate per minute under standard conditions. It was adopted by the International Union of Biochemistry in 1964.

The Role of Co-factors in Enzyme Catalysis

The catalytic activity of many enzymes depends on the presence of small molecules termed *cofactors*, although the precise role varies with the cofactor and the enzyme. Such an enzyme is called a *Conjugated enzyme*. Those not requiring a co factor are called a *Simple enzyme*. Without its cofactor, a conjugated enzyme is referred to as an *apoenzyme*; the complete, catalytically active enzyme is called a *holoenzyme*.

Apoenzyme + cofactor = holoenzyme

Cofactors can be subdivided into two groups: *metals* and *small organic molecules*.

Cofactors that are small organic molecules are called *coenzymes*. Often *derived from vitamins*, coenzymes can be either tightly or loosely bound to the enzyme. If tightly bound, they are called *prosthetic groups*. Loosely associated coenzymes are more like *co substrates* because they bind to and are released from the enzyme just the way substrates and products are.

Enzyme cofactors

Introduction

The functional groups of the amino acid sidechains of enzymes are responsible for many of the catalytic properties of proteins. However, **by themselves, the sidechain functional groups are unable to catalyze all the reactions needed by a cell in metabolism.** For those essential reactions that are impossible or impracticably inefficient within the repertoire of mechanisms catalyzed by side chain functional groups, a small structurally diverse group of molecules known as ***cofactors*** is of central importance.

The catalytic activity of many enzymes depends on the presence *cofactors*, although the precise role varies with the cofactor and the enzyme. Such an enzyme without its cofactor is referred to as an *apoenzyme*; the complete, catalytically active enzyme is called a *holoenzyme*. Hence:

Apoenzyme + Cofactor = Holoenzyme

Cofactors can be subdivided into two groups: **metals** and **small organic molecules**.

Metals can be bound to an enzyme in two possible manners:

a. Tightly and rather permanently: Such enzymes, which carry a tightly bound metal group, are called **Metallozyme**. The metal group present are mostly transition metal ions such as, Fe^{3+}, Fe^{2+}, Mg^{2+}, Zn^{2+}, Co^{3+} etc. one of the earliest recognised metallozyme is Carbonic anhydrase, with Zinc group.

b. Loosely and often transiently: The metal ions in such associations with apoenzymes play the role of activators; hence, they are mostly called **Metal Activators**. They belong to the earth metal group such as, Na^{+}, K^{+}, and Ca^{2+}.

Associated metal cofactors play three roles in enzyme's catalysis.

1. They neutralize negative change and bring about better charge distribution, which can sometimes be necessary for catalysis.
2. They facilitate redox reactions by working as electron acceptor or electron donor.
3. They can promote electrostatic binding between the enzyme and the substrate.

Cofactors that are small organic molecules are called ***coenzymes***. Often derived from vitamins, coenzymes can be either tightly or loosely bound to the enzyme. If tightly bound, they are called ***prosthetic groups***. Loosely associated coenzymes are called **cosubstrate**s because they bind to and are released from the enzyme just as substrates and products are. The use of the same coenzyme by a variety of enzymes and their source in vitamins sets coenzymes apart from normal substrates, however. Enzymes that use the same coenzyme are usually mechanistically similar.

Some examples:

Cofactor	**Enzyme**
Coenzyme	
Thiamine pyrophosphate	Pyruvate dehydrogenase
Flavin adenine nucleotide	Monoamine oxidase
Nicotinamide adenine dinucleotide	Lactate dehydrogenase
Pyridoxal phosphate	Glycogen phosphorylase
Coenzyme A (CoA)	Acetyl CoA carboxylase
Biotin	Pyruvate carboxylase
5′-Deoxyadenosyl cobalamin	Methylmalonyl mutase
Tetrahydrofolate	Thymidylate synthase
Metal	
Zn^{2+}	Carbonic anhydrase
Zn^{2+}	Carboxypeptidase
Mg^{2+}	*Eco*RV
Mg^{2+}	Hexokinase
Ni^{2+}	Urease
Mo	Nitrate reductase
Se	Glutathione peroxidase
Mn^{2+}	Superoxide dismutase

K^+	Propionyl CoA carboxylase

Vitamins as Co-enzymes

Most coenzymes are derived from *vitamins*. Vitamins themselves are organic molecules that are needed in small amounts in the diets of some higher animals. These molecules serve the same roles in nearly all forms of life, but higher animals lost the capacity to synthesize them in the course of evolution. For instance, whereas *E. coli* can thrive on glucose and organic salts, human beings require at least 12 vitamins in the diet. The biosynthetic pathways for vitamins can be complex; thus, it is biologically more efficient to ingest vitamins than to synthesize the enzymes required to construct them from simple molecules. This efficiency comes at the cost of dependence on other organisms for chemicals essential for life.

Vitamins are often classified into 1. **Water soluble** and 2. **Fat soluble**

Water-Soluble Vitamins

Vitamin	Coenzyme	Typical reaction type	Consequences of deficiency
Thiamine (B_1)	Thiamine pyrophosphate	Aldehyde transfer	Beriberi (weight loss, heart problems, neurological dysfunction)
Riboflavin (B_2)	Flavin adenine dinucleotide (FAD)	Oxidation-reduction	Cheliosis and angular stomatitus (lesions of the mouth), dermatitis
Pyridoxine (B_6)	Pyridoxal phosphate	Group transfer to or from amino acids	Depression, confusion, convulsions
Nicotinic acid (niacin)	Nicotinamide adenine dinucleotide (NAD^+)	Oxidation-reduction	Pellagra (dermatitis, depression, diarrhea)
Pantothenic acid	Coenzyme A	Acyl-group transfer	Hypertension
Biotin	Biotin-lysine complexes (biocytin)	ATP-dependent carboxylation and carboxyl-group transfer	Rash about the eyebrows, muscle pain, fatigue (rare)
Folic acid	Tetrahydrofolate	Transfer of one-carbon components; thymine synthesis	Anemia, neural-tube defects in development
B_{12}	5′-Deoxyadenosyl cobalamin	Transfer of methyl groups; intramolecular rearrangements	Anemia, pernicious anemia, methylmalonic acidosis

C (ascorbic acid)		Antioxidant	Scurvy (swollen and bleeding gums, subdermal hemorrhages)

Fat-soluble vitamins

Vitamin	Function	Deficiency
A	Roles in vision, growth, reproduction	Night blindness, cornea damage, damage to respiratory and gastrointestinal tract
D	Regulation of calcium and phosphate metabolism	Rickets (children): skeletal deformaties, impaired growth
		Osteomalacia (adults): soft, bending bones
E	Antioxidant	Inhibition of sperm production; lesions in muscles and nerves (rare)
K	Blood coagulation	Subdermal hemorrhaging

Description of vitamins which act as / give rise to co-enzymes

Not all vitamins are **coenzymes** per se. Some are actually precursors that are transformed into an unabridged coenzyme after further metabolic modification.

1. **Thiamin** is also known as **vitamin B_1** . Thiamin is derived from a substituted pyrimidine and a thiazole which are coupled by a methylene bridge. Thiamin is rapidly converted to its active form, **thiamin pyrophosphate, TPP**, in the brain and liver by a specific enzymes, ***thiamin diphosphotransferase.*** TPP is necessary as a cofactor for the ***pyruvate*** and ***α-ketoglutarate dehydrogenase*** catalyzed reactions as well as the ***transketolase*** catalyzed reactions of the pentose phosphate pathway. A deficiency in thiamin intake leads to a severely reduced capacity of cells to generate energy as a result of its role in these reactions.

2. **Riboflavin** is also known as **vitamin B_2**. Riboflavin is the precursor for the coenzymes, **flavin mononucleotide (FMN)** and **flavin adenine dinucleotide (FAD)**. The enzymes that require FMN or FAD as cofactors are termed flavoproteins. Several flavoproteins also contain metal ions and are termed metalloflavoproteins. Both classes of enzymes are involved in a wide range of redox reactions, e.g. *succinate dehydrogenase* and *xanthine oxidase*. During the course of the enzymatic reactions involving the flavoproteins the reduced forms of FMN and FAD are formed, $FMNH_2$ and $FADH_2$, respectively.

3. **Niacin** (nicotinic acid and nicotinamide) is also known as **vitamin B_3**. Both nicotinic acid and nicotinamide can serve as the dietary source of vitamin B_3. Niacin is required for the synthesis of the active forms of vitamin B_3, **nicotinamide adenine dinucleotide (NAD^+)** and **nicotinamide adenine dinucleotide phosphate ($NADP^+$)**. Both NAD^+ and $NADP^+$ function as cofactors for numerous dehydrogenase, e.g., *lactate* and *malate dehydrogenases*. Niacin is not a true vitamin in the strictest definition since it can be derived from the amino acid tryptophan. However, the ability to utilize tryptophan for niacin synthesis is inefficient (60 mg of tryptophan are required to synthesize 1 mg of

niacin). Also, synthesis of niacin from tryptophan requires vitamins B_1, B_2 and B_6 which would be limiting in themselves on a marginal diet.

4. **Pantothenic acid** is also known as **vitamin B_5**. Pantothenic acid is formed from □-alanine and pantoic acid. Pantothenate is required for synthesis of coenzyme A, CoA and is a component of the acyl carrier protein (ACP) domain of fatty acid synthase. Pantothenate is, therefore, required for the metabolism of carbohydrate via the TCA cycle and all fats and proteins. At least 70 enzymes have been identified as requiring CoA or ACP derivatives for their function.

5. **Pyridoxal, pyridoxamine** and **pyridoxine** are collectively known as **vitamin B_6**. All three compounds are efficiently converted to the biologically active form of vitamin B_6, **pyridoxal phosphate**. This conversion is catalyzed by the ATP requiring enzyme, ***pyridoxal kinase***. Pyridoxal phosphate functions as a cofactor in enzymes involved in transamination reactions required for the synthesis and catabolism of the amino acids as well as in glycogenolysis as a cofactor for ***glycogen phosphorylase***.

6. **Biotin** is the cofactor required of enzymes that are involved in carboxylation reactions, e.g. ***acetyl-CoA carboxylase*** and ***pyruvate carboxylase***.

7. **Cobalamin** is more commonly known as **vitamin B_{12}**. Vitamin B_{12} is composed of a complex tetrapyrrol ring structure (corrin ring) and a cobalt ion in the center. Vitamin B_{12} is synthesized exclusively by microorganisms. There are only two physiologically significant reactions in an organism that require vitamin B_{12} as a cofactor. During the catabolism of fatty acids with an odd number of carbon atoms and the amino acids valine, isoleucine and threonine the resultant propionyl-CoA is converted to succinyl-CoA for oxidation in the TCA cycle. One of the enzymes in this pathway, ***methylmalonyl-CoA mutase***, requires vitamin B_{12} as a cofactor in the conversion of methylmalonyl-CoA to succinyl-CoA. The 5'-deoxyadenosine derivative of cobalamin is required for this reaction. The second reaction requiring vitamin B_{12} catalyzes the conversion of homocysteine to methionine and is catalyzed by ***methionine synthase***. This reaction results in the transfer of the methyl group from N^5-methyltetrahydrofolate to hydroxycobalamin generating tetrahydrofolate (THF) and methylcobalamin during the process of the conversion.

8. **Folic acid** is a conjugated molecule consisting of a pteridine ring structure linked to para-aminobenzoic acid (**PABA**) that forms pteroic acid. Folic acid itself is then generated through the conjugation of glutamic acid residues to pteroic acid. Folic acid is obtained primarily from yeasts and leafy vegetables as well as animal liver. Animal cannot synthesize PABA nor attach glutamate residues to pteroic acid, thus, requiring folate intake in the diet. Folic acid is reduced within cells to tetrahydrofolate (THF also H_4folate) through the action of ***dihydrofolate reductase*** **(DHFR)**, an NADPH-requiring enzyme.

   The function of THF derivatives is to carry and transfer various forms of one carbon units during biosynthetic reactions. The one carbon units are either methyl, methylene, methenyl, formyl or formimino groups. These one carbon transfer reactions are required in the biosynthesis of serine, methionine, glycine, choline and the purine nucleotides and dTMP.

   The ability to acquire choline and amino acids from the diet and to salvage the purine nucleotides makes the role of N^5,N^{10}-methylene-THF in dTMP synthesis the most metabolically significant function for this vitamin. The role of vitamin B_{12} and N^5-methyl-THF in the conversion of homocysteine to methionine also can have a significant impact on the ability of cells to regenerate needed THF.

9. **Ascorbic acid** is more commonly known as **vitamin C**. Ascorbic acid is derived from glucose via the uronic acid pathway. The main function of ascorbate is as a reducing agent in a number of different reactions. Vitamin C has the potential to reduce cytochromes a and c of the respiratory chain as well as molecular oxygen. The most important reaction requiring ascorbate as a cofactor is the hydroxylation of proline residues in collagen. Vitamin C is, therefore, required for the maintenance of normal connective tissue as well as for wound healing since synthesis of connective tissue is the first event in wound tissue remodeling. Vitamin C also is necessary for bone remodeling due to the presence of collagen in the organic matrix of bones.

   Several other metabolic reactions require vitamin C as a cofactor. These include the catabolism of tyrosine and the synthesis of epinephrine from tyrosine and the synthesis of the bile acids. It is also believed that vitamin C is involved in the process of steroidogenesis since the adrenal cortex contains high levels of vitamin C which are depleted upon adrenocorticotropic hormone (ACTH) stimulation of the gland.

   Deficiency in vitamin C leads to the disease **scurvy** due to the role of the vitamin in the post-translational modification of collagens.

10. **Vitamin A** consists of three biologically active molecules, **retinol**, **retinal** (retinaldehyde) and **retinoic acid**. Each of these compounds are derived from the plant precursor molecule, **b-carotene** (a member of a family of molecules known as **carotenoids**). Within cells both retinol and retinoic acid bind to specific receptor proteins. Following binding, the receptor-vitamin complex interacts with specific sequences in several genes involved in growth and differentiation and affects expression of these genes. In this capacity retinol and retinoic acid are considered hormones of the steroid/thyroid hormone superfamily of proteins. Photoreception in the eye is the function of two specialized cell types located in the retina; the rod and cone cells. Both rod and cone cells contain a photoreceptor pigment in their membranes. The photosensitive compound of most mammalian eyes is a protein called **opsin** to which is covalently coupled an aldehyde of vitamin A. The opsin of rod cells is called **scotopsin**. The photoreceptor of rod cells is specifically called **rhodopsin** or **visual purple**. This compound is a complex between scotopsin and the 11-*cis*-retinal (also called 11-*cis*-retinene) form of vitamin A. Rhodopsin is a serpentine receptor imbedded in the membrane of the rod cell. Coupling of 11-*cis*-retinal occurs at three of the transmembrane domains of rhodopsin. Intracellularly, rhodopsin is coupled to a specific G-protein called **transducin**.

Role of co-enzymes in catalysis

Together with enzymes, **coenzymes** provide special chemical properties that permit exceptional chemical reactivities and reactions not otherwise possible at physiologic conditions. The **role** that **coenzymes** play in biochemistry is so extraordinary and so pivotal, it led **David Metzler** to call them "*Nature's Special Reagents.*"

Coenzymes often serve as additional reagents in enzyme-catalyzed reactions, as stabilizers, or carriers of functional groups, protons, or electrons. Humans are unable to synthesize most of the **coenzymes** from elementary precursors, and if a dietary source is not available a deficiency with obvious clinical symptoms arises.

The functional role of coenzymes is to act as transporters of chemical groups from one reactant to another. The chemical groups carried can be as simple as the hydride ion ($H^+ + 2e^-$) carried by NAD or the mole of hydrogen carried by FAD; or they can be even more complex than the amine ($-NH_2$) carried by pyridoxal phosphate.

Since coenzymes are chemically changed as a consequence of enzyme action, it is often useful to consider coenzymes to be a special class of substrates, or **second substrates**, which are common to many different holoenzymes. In all cases, the coenzymes donate the carried chemical grouping to an acceptor molecule and are thus regenerated to their original form. This regeneration of coenzyme and holoenzyme fulfills the definition of an enzyme as a chemical catalyst, since (unlike the usual substrates, which are used up during the course of a reaction) coenzymes are generally regenerated.

The modes of action made possible by **coenzymes** can be considered to fall into three mechanistic groupings.

1. They can participate in **group transfer** reactions by providing energy and/or donating part of the coenzyme molecule to another enzyme substrate. A good example of this is **ATP** which is a provider of energy to drive many reactions, but it is also source of **phosphate**, **pyrophosphate**, **phosphoribosyl**, and **adenosyl** moieties to other reactants of a given reaction. The transfer of one carbon units by **tetrahydrofolate** also puts it in this class.
2. A second category of **coenzymes** reacts at the active site of the enzyme to **modify the structure of the substrate** so as to facilitate the conversion of substrate to product. Examples in this grouping include **CoA**, **thiamine**, **pyridoxal phosphate** and **5'deoxyadenosylcobalamin (B12)**.
3. The third class consists of those **coenzymes** that participate **in redox reactions** by serving as carriers of electrons or protons. **Coenzymes** in this group include **ascorbic acid**, **FAD**, **FMN**, and the **pyridine nucleotides: NAD^+, NADH, $NADP^+$,** and **NADPH**.

Enzyme Inhibition

Introduction

The activity of many enzymes can be inhibited by the binding of specific small molecules and ions. This means of inhibiting enzyme activity serves as a major control mechanism in biological systems.

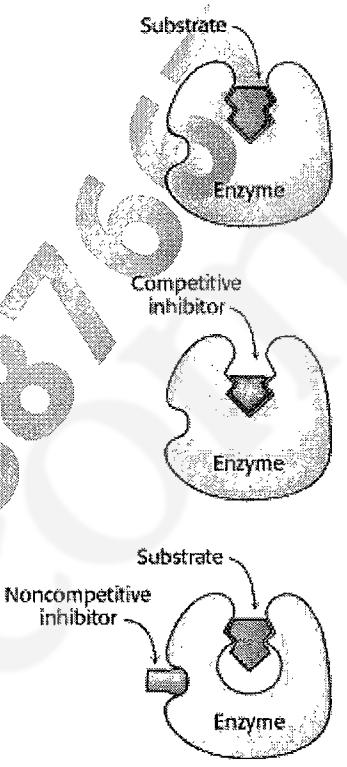
Enzyme inhibition is phenomenon in which the binding of a chemical substance to the enzyme reduces the catalytic activity of the enzyme. The substances which cause inhibition are called enzyme inhibitors.

Types of Enzyme Inhibition

Enzyme inhibition can be either reversible or irreversible.

1. An ***irreversible inhibitor*** dissociates very slowly from its target enzyme because it has become tightly bound to the enzyme, either covalently or noncovalently. If the inhibitor binds permanently to the enzyme, it is then termed Inactivator. Some irreversible inhibitors are important drugs. Penicillin acts by covalently modifying the enzyme transpeptidase, thereby preventing the synthesis of bacterial cell walls and thus killing the bacteria. Aspirin acts by covalently modifying the enzyme cyclooxygenase, reducing the synthesis of inflammatory signals. DIPF (diisopropylphosphofluoridate) is an example of group-specific irreversible inhibitor. It modifies only 1 of the 28 serine residues in the proteolytic enzyme chymotrypsin and also in acetylcholinesterase, an enzyme important in the transmission of nerve impulses. Thus, DIPF and similar compounds that bind and inactivate acetylcholinesterase are potent nerve gases.
2. ***Reversible inhibition***, in contrast with irreversible inhibition, is characterized by a rapid dissociation of the enzyme-inhibitor complex.

There are three subtypes of reversible enzyme inhibition. (As explained by Voet, Voet & Pratt in Fundamentals of Biochemistry, 2nd Edition, 2006).



A. In ***competitive inhibition***, an enzyme can bind substrate (forming an ES complex) or inhibitor (EI) but not both (ESI). The competitive inhibitor resembles the substrate and binds to the active site of the enzyme. In *competitive inhibition*, the inhibitor competes with the substrate for the active site. The substrate is thereby prevented from binding to the same active site. *A competitive inhibitor diminishes the rate of catalysis by reducing the proportion of enzyme molecules bound to a substrate.* It thus increases the value of K_M but does not affect V_{Max}. At any given inhibitor concentration, competitive inhibition can be relieved by increasing the substrate concentration. Under these conditions, the substrate "outcompetes" the inhibitor for the active site. Methotrexate is a structural analog of tetrahydrofolate, a coenzyme for the enzyme dihydrofolate reductase, which plays a role in the biosynthesis of purines and pyrimidines. It binds to dihydrofolate reductase 1000-fold more tightly than the natural substrate and inhibits nucleotide base synthesis. It is used to treat cancer. Malonate is an inhibitor of the TCA Cycle Enzyme Succinate Dehydronase, because it structurally resembles Succinate.

B. In ***uncompetitive inhibition***, the inhibitor binds to the enzyme substrate complex but not to the free enzyme. In *uncompetitive inhibition*, which also is reversible, the inhibitor and substrate can bind simultaneously to an enzyme molecule at different binding sites. A noncompetitive inhibitor acts by decreasing the turnover number (catalytic activity) rather than by diminishing the proportion of enzyme molecules that are bound to substrate. In essence, the inhibitor simply lowers the concentration of functional enzyme. The remaining enzyme behaves like a more dilute solution of enzyme; V_{max} is lower, but K_M is the same. Noncompetitive inhibition cannot be overcome by increasing the substrate concentration.

C. A more complex pattern, called ***mixed inhibition***, is produced when a single inhibitor both hinders the binding of substrate and decreases the turnover number of the enzyme. This type of inhibition is also called *non-competitive* inhibition. It can be presumed that mixed inhibitors bind to such sites of the enzyme, which are important both for substrate binding as well as for catalytic action. The mixed inhibition thus increases the value of K_M and also lowers the V_{Max}.

Transition-State Analogs are potent inhibitors of enzymes. Transition-State Analogs are compounds resembling the transition state of a catalyzed reaction. There are certain enzymes which carry out catalysis by *selective binding of the transition state.* Only such enzymes can be inhibited by Transition-State Analogs. The inhibition of proline racemase is a good example. The racemization of proline proceeds through a transition state in which the tetrahedral α- carbon atom has become trigonal by loss of a proton. The inhibitor pyrrole 2-carboxylate binds to the racemase 160 times as tightly as does proline. *The α-carbon atom of this inhibitor, like that of the transition state, is trigonal.*

Significance of Enzyme Inhibition

1. Inhibiting enzyme activity serves as a major control mechanism in biological systems. The regulation of allosteric enzymes typifies this type of control.
2. Many drugs and toxic agents act by inhibiting enzymes. For example, Penicillin acts by covalently modifying the enzyme transpeptidase, thereby preventing the synthesis of bacterial cell walls and thus killing the bacteria. Aspirin acts by covalently modifying the enzyme cyclooxygenase, reducing the synthesis of inflammatory signals. The toxin from *Vibrio cholerae*, the causative agent of cholera, disables sensor-response pathways in intestinal epithelial cells by ADP-ribosylating the GTP-binding proteins (G-proteins) that link cell surface receptors to adenylyl cyclase. The consequent activation of the cyclase triggers the flow of water into the intestines, resulting in massive diarrhea and dehydration.
3. Inhibition by particular chemicals can be a source of insight into the mechanism of enzyme action: specific inhibitors can often be used to identify residues critical for catalysis. For example, DIPF modifies only 1 of the 28 serine residues in the proteolytic enzyme chymotrypsin, implying that this serine residue is especially reactive. Similarly, the inhibitory power of transition-state analogs highlights a particular strategy of catalysis: selective binding of the transition state.

Enzyme Regulation

Introduction

In living cells, there are hundreds of different enzymes working together in a coordinated manner. Living cells neither synthesize nor breakdown more material than what is required for normal metabolism and growth. All of this necessitates precise **control mechanisms** for turning metabolic reactions on and off.

A careful investigation of a cell reveals that enzymes are carefully controlled for their presence and activity both. Thus, enzymes can be controlled or regulated in two ways: **1. controlling the synthesis of the enzyme** (pre-translational control) and **2. controlling the activity of the enzyme** (post-translational control).

Pre-translational control

Pre-translational control refers to the control of enzyme synthesis. For ribozymes (RNA based enzymes) the term translation does not apply as a step in synthesis. Hence, their pre-synthesis control can be exerted either at the level of Transcription or Post-transcription modifications. Essentially, pre-synthesis control of enzymes is a strategy to regulate their abundance in the cell at a given point of time and actually *involves regulation of gene expression*.

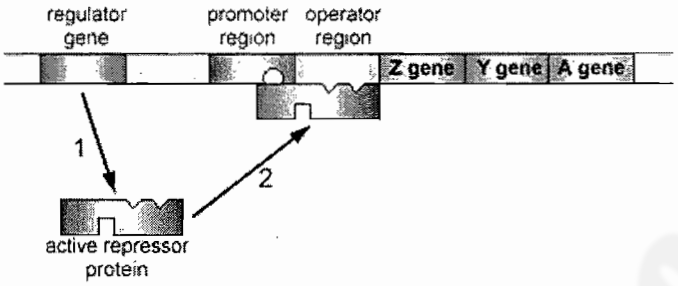
One of the classic examples of enzyme synthesis occurring at a time when they are actually required can be seen in Operon circuits of Prokaryotes. In prokaryotic cells, this **involves the induction or repression of enzyme synthesis by regulatory proteins that can bind to DNA and either block or enhance the function of RNA polymerase**, the enzyme required for transcription. The regulatory proteins are part of either an operon or a regulon. An **operon** is a set of genes transcribed as a polycistronic message that is collectively controlled by a regulatory protein.

An example of this is the *lac* **operon** that encodes for the three enzymes needed for the degradation of lactose by *E. coli*. *E.coli* will only sunthesize the three enzymes it requires to utilize lactose if that sugar is present in the surrounding environment. In this case, **lactose functions as an inducer**. In the absence of lactose, the

repressor protein binds to the operator and RNA polymerase is unable to get beyond the operator and transcribe the genes for utilization of lactose and the three enzymes for degradation of lactose are not synthesized. When lactose, the inducer, is present, it binds to the allosteric repressor protein and causes it to change shape in such a way that it is no longer able to bind to the operator. Now RNA polymerase can transcribe the three genes required for the degradation of lactose and the bacterium is able to synthesize the enzymes needed for its utilization.

The Lactose Operon

Step 1.



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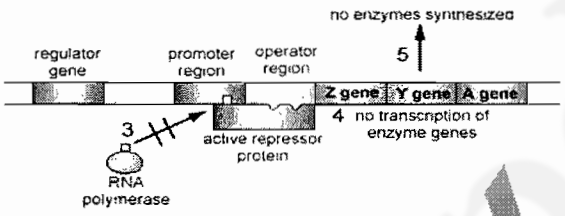

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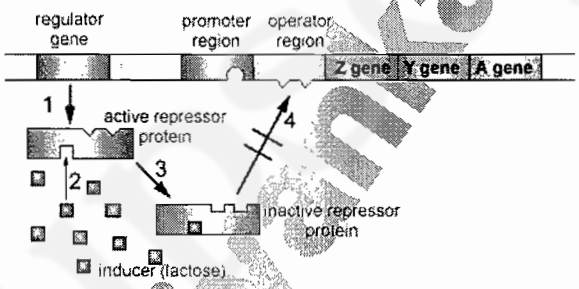

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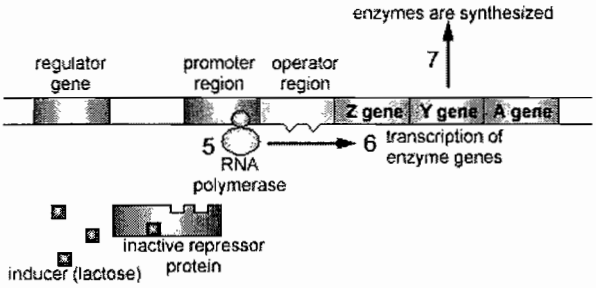


The advantage of control of enzymes at the level of synthesis is economy of ATP/GTP that would have otherwise been wastefully spent during various steps of gene expression. However, it is often not a very swift response to correlate enzyme action to cellular requirements.

Post-translational control

Post-translational control is certainly a rapid means to controlling the activity of the enzyme according to cellular requirements. This is often linked to • intra-cellular signals, • extra-cellular signals, and • process flow or progress for achieving precise regulation. The variety of Post-translational control of enzyme action include:

1. Compartmentalization
2. Allostery

   Two types:

   A. Homotropic

   B. Heterotropic
3. Feedback Control
4. Covalent modification

   Two types:

   A. Reversible

   B. Proteolytic cleavage [irreversible]
5. Regulatory protein attachment
6. Protein breakdown

Compartmentalization

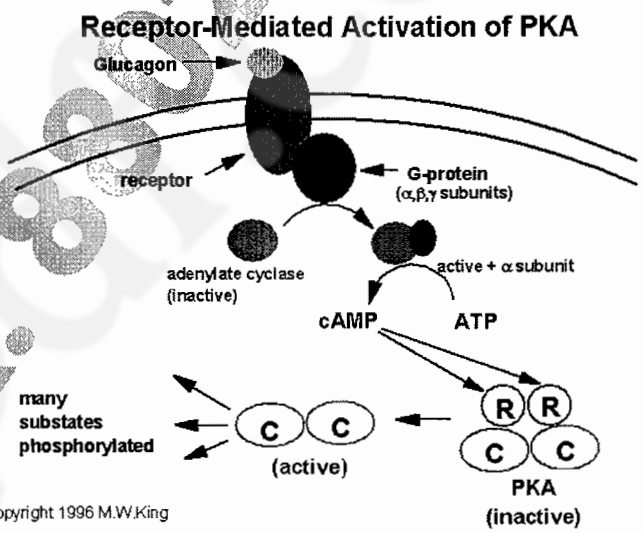
A primary mechanism of regulating enzyme activity is to sequester enzymes in compartments where access to their substrates is limited. For example, the proteolysis of cell proteins and glycolipids by enzymes responsible for their degradation is controlled by sequestering these enzymes within the lysosome. Appropriate compartmentalization of enzymes is achieved through various mechanisms of protein targeting. This makes sure that an enzymes is found only at the required subcellular location and nowhere else.

Allostery

In addition to simple enzymes that interact only with substrates and inhibitors, there is a class of enzymes that bind small, physiologically important molecules and modulate activity in ways other than those described above. These are known as **allosteric enzymes**; the small regulatory molecules to which they bind are known as **effectors**. Allosteric effectors bring about catalytic modification by binding to the enzyme at distinct allosteric sites, well removed from the catalytic site, and causing conformational changes that are transmitted through the bulk of the protein to the catalytically active site(s).

The hallmark of effectors is that when they bind to enzymes, they alter the catalytic properties of an enzyme's active site. Those that increase catalytic activity are known as positive effectors. Effectors that reduce or inhibit catalytic activity are negative effectors.

Most allosteric enzymes are oligomeric (consisting of multiple subunits); generally they are located at or near branch points in metabolic pathways, where they are influential in directing substrates along one or another of the available metabolic paths. The effectors that modulate the activity of these allosteric enzymes are of two types. Those activating and inhibiting effectors that bind at allosteric sites are called **heterotropic effectors**. (Thus there exist both positive and negative heterotropic effectors.) These effectors can assume a vast diversity of chemical forms, ranging from simple inorganic molecules to complex nucleotides such as cyclic adenosine monophosphate (cAMP). Their single defining feature is that they are not identical to the substrate.



In many cases the substrate itself induces distant allosteric effects when it binds to the catalytic site. Substrates acting as effectors are said to be **homotropic effectors**. When the substrate is the effector, it can act as such, either by binding to the substrate-binding site, or to an allosteric effector site. When the substrate binds to the catalytic site it transmits an activity-modulating effect to other subunits of the molecule. Often used as the model of a homotropic effector is **O_2 in relation to hemoglobin**, where O_2 binding at one of the active sites enhances O_2 binding at other active sites too.

For the presence of cooperation in substrate binding alloasteric enzymes do not follow the Michaelis-Menton Kinetic model; they rather show a sigmoidal curve of enzyme rates.

There are two ways that enzymatic activity can be altered by effectors: the V_{max} can be increased or decreased, or the K_m can be raised or lowered. Enzymes whose K_m is altered by effectors are said to be **K-type enzymes** and the effector a K-type effector. If V_{max} is altered, the enzyme and effector are said to be V-type. Many allosteric enzymes respond to multiple effectors with V-type and K-type behavior. Here again, hemoglobin is often used as a model to study allosteric interactions, although it is not strictly an enzyme.

In the previous examples, allosteric sites and catalytic sites were homogeneously present on every subunit of an allosteric enzyme. While this is often the case, there is another class of allosteric enzymes that are comprised of separate catalytic and regulatory subunits. The archetype of this class of enzymes is *cAMP-dependent protein kinase* (PKA), whose mechanism of activation is illustrated here. The enzyme is tetrameric,

containing two catalytic subunits and two regulatory subunits, and enzymatically inactive. When intracellular cAMP levels rise, one molecule of cAMP binds to each regulatory subunit, causing the tetramer to dissociate into one regulatory dimer and two catalytic monomers. In the dissociated form, the catalytic subunits are fully active; they catalyze the phosphorylation of a number of other enzymes, such as those involved in regulating glycogen metabolism. The regulatory subunits have no catalytic activity.

Feedback Control

If the product of a series of enzymatic reactions, e.g., an amino acid, begins to accumulate within the cell, it may specifically inhibit the action of the first enzyme involved in its synthesis. Thus further production of the enzyme is halted.

The activity of almost every enzyme is a cell is regulated by feedback inhibition. Feedback inhibition is an example of common biological control mechanism called negative feedback. When the product is in abundance, it binds competitively or non-competitively with its enzyme's active site, and as the product is used up, inhibition is reduced and more product can be produced. In this way the concentration of the product is always controlled within a certain range.

Most enzymatic pathways are also regulated by feedback inhibition, but in these cases the end product of the pathway binds at an allosteric site on the first enzyme of the pathway. This binding shuts down the pathway, and not more product id produced. The reaction series converting theronine to isoleucine is a classic exampleof allosteric regulation. Five enzymes acting in sequence catalyze the pathway. The final product of the sequence, isoleucine, acts as an inhibitor of the first enzyme of the pathway, threonine deaminase. As the pathway produces isoleucine, any molecules made in excess of cell requirements combine reversibly with threonine deaminase at a location outside the active site. The combination converts threonine deaminase to the T state and inhibits its ability to combine with threonine. The pathway is then turned off. If the concentration of isoleucine later falls as a result of its use in cell synthesis, isoleucine releases from the threonine deaminase enzymes, converting them to the R state in which they have high affinity of the substrate, conversion of threonine to isoleucine takes place.

Covalent modification

A. Reversible

The reversible covalent attachment of another molecule can modify the activity of enzymes and many other proteins. In these instances, a donor molecule provides a functional moiety that modifies the properties of the enzyme. Reversible covalent modification is a major mechanism for the rapid and transient regulation of enzyme activity. The best examples, again, come from studies on the regulation of glycogen metabolism where phosphorylation of ***glycogen synthase*** and ***glycogen phosphorylase kinase*** results in the stimulation of glycogen degradation while glycogen synthesis is coordinately inhibited. Numerous other enzymes of intermediary metabolism are affected by phosphorylation, either positively or negatively. These covalent phosphorylations can be reversed by a separate sub-subclass of enzymes known as ***phosphatases***. Recent research has indicated that the aberrant phosphorylation of growth factor and hormone receptors, as well as of proteins that regulate cell division, often leads to unregulated cell growth or cancer. The usual sites for phosphate addition to proteins are the serine, threonine and tyrosine R group hydroxyl residues. **Phosphorylation and dephosphorylation are the most common** [as also seen in **signal cascading** and **cell cycle regulation**] but not the only means of covalent modification.

Histones—proteins that assist in the packaging of DNA into chromosomes as well as in gene regulation—are rapidly **acetylated** and **deacetylated** in vivo. In addition to this reversible **adenylation**, **methylation** and **ribosylation** of enzymes at specific domains are also carried out frequently to control their activity.

B. Irreversible

Certain enzymes need a permanent group transfer in order to be activated; such as RUBISCO need carbamylation and several human digestive enzymes need sulphonation for attaining functionally active state.

Another strategy to control enzyme action is **proteolytic cleavge** of the enzymatic precursors. Proenzyme activation is a more rapid method of increasing enzyme activity but, as a regulatory mechanism, it has the disadvantage of not being a reversible process. Proenzymes are generally synthesized in abundance, stored in secretory granules and covalently activated upon release from their storage sites. Examples of important proenzymes include **pepsinogen**, **trypsinogen** and **chymotrypsinogen**, which give rise to the proteolytic digestive enzymes. Likewise, many of the proteins involved in the cascade of chemical reactions responsible for blood clotting are synthesized as proenzymes. Other important proteins, such as peptide hormones and collagen, are also derived by covalent modification of precursors.

Regulatory protein attachment

Regulatory protein attachment is less frequently seen but it is quite a critical mechanism to control enzyme activity where it is found. 2 examples illustrate this.

1. Activation of CDKs by binding of Cyclins during the cell cycle control
2. Activation of several cytosolic enzymes by binding of Calmodulin

In addition, there is a wide variety of enzyme activators and repressor in the cell which are proteinaceous in nature.

Protein breakdown

Usually enzymes are broken down after they have served their catalytic purposes. The normal breakdown pathway is by **Ubiquitinization** and subsequent targeting to the **proteosome complex**. This mechanism maintains, like pre-synthesis control, the optimal abundance of enzymes in the cell according to the requirements.

Plant Hormones- I: Auxins

Introduction to Auxins

Auxin (Greek, *Auxein* = to grow) is a generic term for phytohormonal compounds (generally acids with an unsaturated cyclic nucleus or derivatives of such acids) characterized by their capacity to induce elongation in shoot cells, and tropisms in roots and stems. Auxins resemble Indole 3-acetic acid (IAA) in physiological action. They are highly versatile phytohormones and affect many other processes beides elongation, but elongation is considered critical in Auxin characterization.

Clealand's concept of Auxins

Clealand in 1996, proposed a comprehensive concept of Auxins. According to this concept:

An Auxin is a compound that has a spectrum of biological activities similar to, but not necessarily the same, with IAA. This includes the ability to -

- Induce cell elongation in isolated coleoptile or stem sections
- Induce cell division in callus tissue culture in the presence of a cytokinin
- Promote lateral root formation at the cut surface of stems
- Induce parthenocarpic tomato fruit growth
- Induce ethylene formation

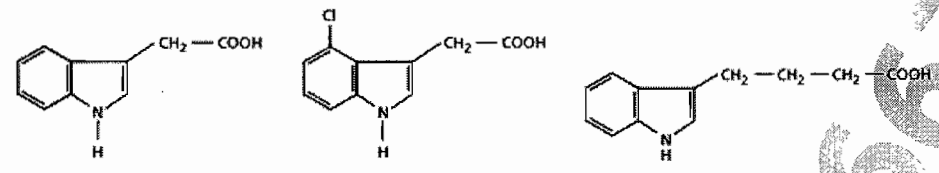
Auxin was the first growth hormone discovered in plants in late 1800s. Along with Cytokinins, Auxins form the essential hormone system in a plant. So far, no plant has been shown to survive without these two hormones.

The broad range of Auxin activity includes the following:

- Cell enlargement of stems, leaves and roots
- Tropisms of stems and roots
- Fruit set, fruit growth and embryo growth
- Apical dominance
- Cell and organ differentiation
- Flower initiation and development
- Abscission of leaves
- Parthenocarpy in some plants
- Enlargement and cell division of callus tissue culturesChemical Nature

The chemical nature of Auxins was determined by **F. Kögl** and **AJ Hagen-Smit**. The substance isolated by them from human urine that produced Auxin actions was **Indole 3-acetic acid [IAA].** IAA is the principal naturally occurring Auxin in almost all the higher plants and fungi.

There are two other universally occurring natural Auxins, apart from IAA. They are 4-chloro-indoleacetic acid and indole-3-butyric acid (IBA).

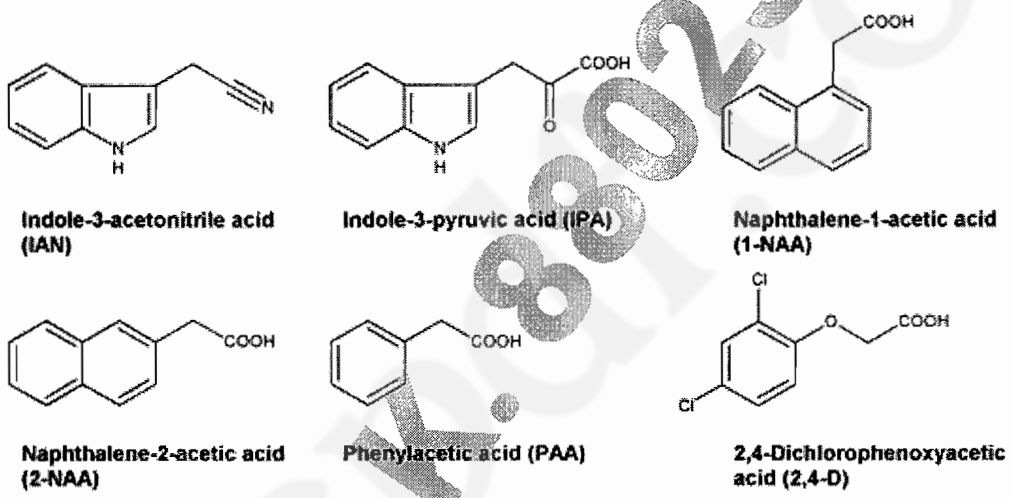


Indole-3-acetic acid (IAA) | 4-Chloroindole-3-acetic acid (4-Cl-IAA) | Indole-3-butyric acid (IBA)

Figure 5: Structure of three naturally occurring Auxins

All the Auxins studied so far are either acids with an unsaturated cyclic nucleus or derivatives of such acids.

Owing to a relatively simple structural backbone, a large number of molecules with Auxin activity have been synthesized in various labs. Some major synthetic Auxins are given in the following diagram.



Indole-3-acetonitrile acid (IAN) | Indole-3-pyruvic acid (IPA) | Naphthalene-1-acetic acid (1-NAA)

Naphthalene-2-acetic acid (2-NAA) | Phenylacetic acid (PAA) | 2,4-Dichlorophenoxyacetic acid (2,4-D)

Figure 6: Major synthetic Auxins

Synthesis of Auxins

Location of synthesis

IAA biosynthesis is associated with rapidly dividing and rapidly growing tissues, especially in shoots. Although virtually all plant tissues appear to be capable of producing low levels of IAA, **shoot apical meristems, young leaves, and developing fruits and seeds are the primary sites of IAA synthesis**.

Synthesis pathways

Multiple pathways exist for the biosynthesis of IAA. IAA is structurally related to the amino acid tryptophan, and early studies on Auxin biosynthesis considered tryptophan as the probable precursor. However, current evidences show that plants convert tryptophan to IAA by several pathways, and there are some other pathways also.

Some important pathways of Auxin synthesis are as follows.

The IPA pathway. The indole-3-pyruvic acid (IPA) pathway is probably the most common of the tryptophan-dependent pathways. It involves a deamination reaction to form IPA, followed by a decarboxylation reaction to form indole-3-acetaldehyde (IAid). Indole-3-acetaldehyde is then oxidized to IAA by a specific dehydrogenase.

The TAM pathway. The tryptamine (TAM) pathway is similar to the IPA pathway, except that the order of the deamination and decarboxylation reactions is reversed, and different enzymes are involved. Species that do not utilize the IPA pathway possess the TAM pathway. In at least one case (tomato), there is evidence for both the IPA and the TAM pathways.

The IAN pathway. In the indole-3-acetonitrile (IAN) pathway, tryptophan is first converted to indole-3-acetaldoxime and then to indole-3-acetonitrile. The enzyme that converts IAN to IAA is called *nitrilase*. The IAN pathway may be important in only three plant families: the Brassicaceae (mustard family), Poaceae (grass family), and Musaceae (banana family). Nevertheless, nitri- lase-like genes or activities have recently been identified in the Cucurbitaceae (squash family), Solanaceae (tobacco family), Fabaceae (legumes), and Rosaceae (rose family).

The IAM pathway. This is also a tryptophan-dependent biosynthetic pathway that uses indole-3-acetamide (IAM) as an intermediate. It is used by various pathogenic bacteria, such as *Pseudomonas savastanoi* and *Agrobacterium tumefaciens*.

Tryptophan independent pathway. In addition to the tryptophan-dependent pathways, recent genetic studies have provided evidence that plants can synthesize IAA via one or more tryptophan-independent pathways. The most striking of these studies in maize involves the *orange pericarp* (*orp*) mutant, in which both subunits of the enzyme tryptophan synthase are inactive. Despite the block in tryptophan biosynthesis, the *orp* mutant contains amounts of IAA 50-fold higher than those of a wild-type plant. Such pathways probably use indole or its precursor, indole-3- glycerol phosphate in IAA biosynthesis. But, the immediate precursor of IAA in the tryptophan-independent pathway has not yet been identified.

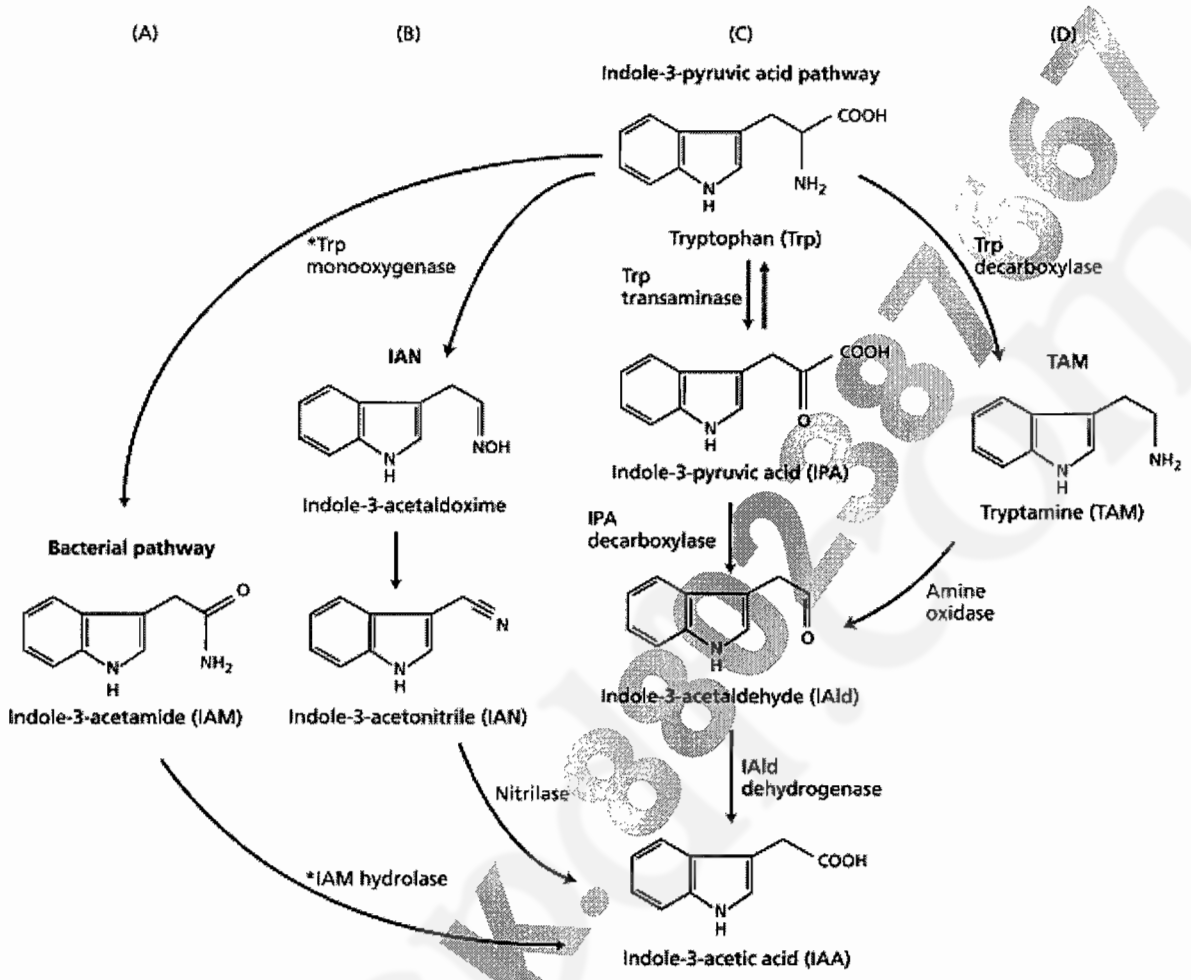


Figure 7: Tryptophan-dependent pathways of IAA biosynthesis in plants and bacteria

Auxin Transport

Auxin moves through the plant by two mechanisms.

- It passes in the sap moving through the phloem from where it is synthesized (its "source", usually the shoot) to a "sink" (e.g., the root).
- It also passes from cell to cell by the following mechanism. : Auxin can enter the cell by diffusion and also through **influx** transporters in the plasma membrane. It moves out through efflux transporters — called **PIN proteins**. Eight different types of PIN proteins have been identified so far.

Auxin action

Mechanism of action

Auxin effects are mediated by two different pathways:

1. Immediate, direct effects on the cell
2. Turning on of new patterns of gene expression

Direct effects of Auxin

The arrival of Auxin in the cytosol initiates immediate responses such as:

- Changes in the concentration of and movement of ions in and out of the cell
- Reduction in the redistribution of pin proteins

Some of the direct effects of Auxin may be mediated by its binding to a cell-surface receptor designated **ABP1** (Auxin-binding protein 1).

Effects of Auxin on gene expression

Many Auxin effects are mediated by changes in the transcription of genes. The steps are as follows:

Auxin enters the nucleus and binds to its receptor, a protein called **TIR1** which now can bind to proteins responsible for attaching ubiquitin to one or more **Aux/IAA proteins**.
This triggers the destruction of the Aux/IAA proteins by proteasomes.
Aux/IAA proteins normally bind transcription factors called Auxin response factors (ARF) preventing them from activating the promoters and other control sequences of genes that are turned on (or off) by Auxin.
Destruction of the Aux/IAA proteins relieves this inhibition, and gene transcription begins.

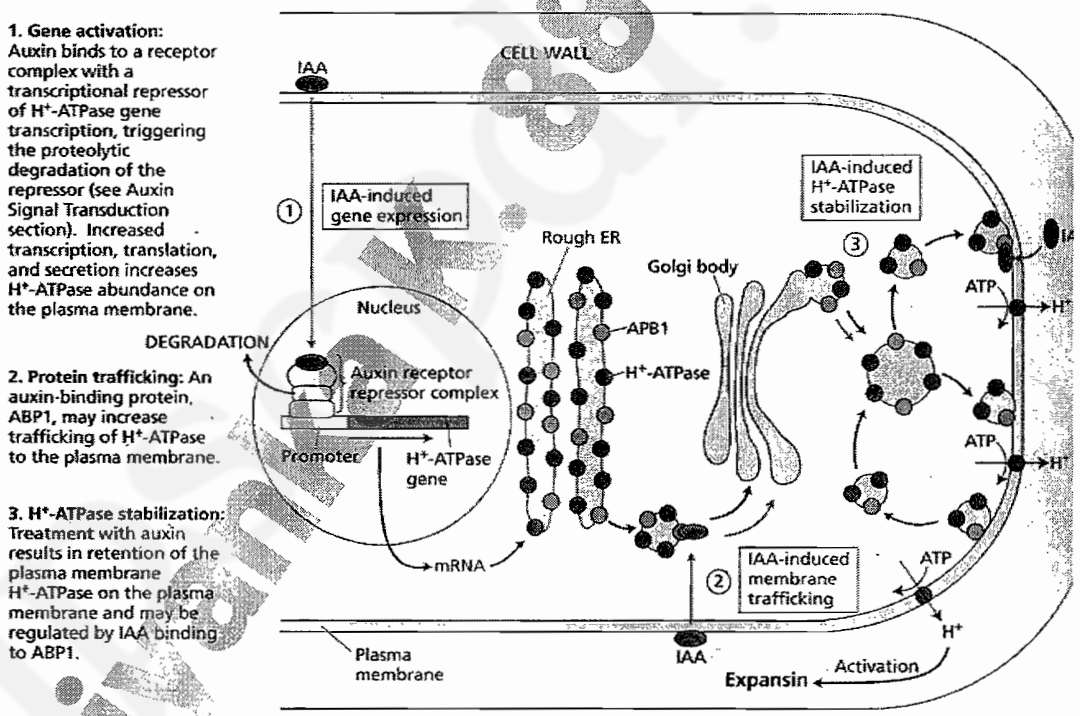


Figure 8: Action of Auxin on Gene Expression (Some cytosolic actions also shown)

Range of physiological responses of Auxins

Cell expansion

According to the widely accepted acid growth hypothesis, hydrogen ions act as the intermediate between Auxin and cell wall loosening. The source of the hydrogen ions is the plasma membrane H+-ATPase, whose activity is thought to increase in response to Auxin.

At acidic pH values, expansins loosen cell walls by weakening the hydrogen bonds between the polysaccharide components of the wall.

Auxin increases the rate of proton extrusion by two possible mechanisms:

1. Activation of preexisting plasma membrane H^+-ATPases (**Figure 5**, see below)
2. Synthesis of new H+-ATPases on the plasma membrane (**Figure 4**, see on the previous page)

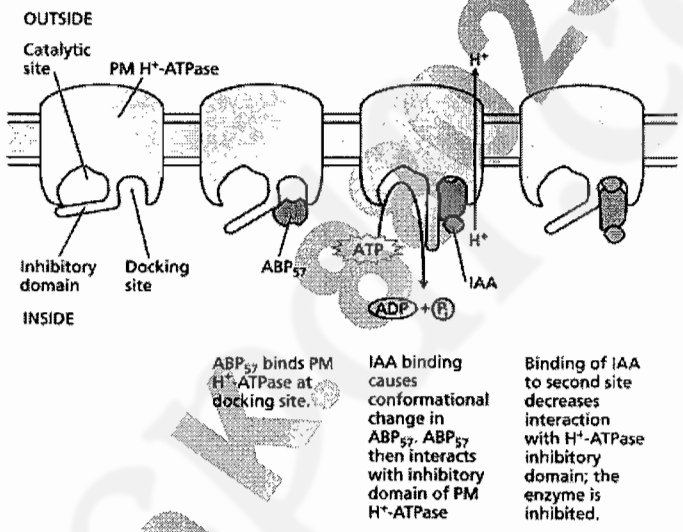


Figure 9: Activation of preexisting plasma membrane H^+-ATPases

Leaf Formation

The formation of new leaves in the apical meristem is initiated by the accumulation of Auxin. Already-developing leaves deplete the surrounding cells of Auxin so that the new leaves do not form too close to them. In this way, the characteristic pattern of leaves in the plant is established. Auxin also controls the precise patterning of the epidermal cells of the developing leaf.

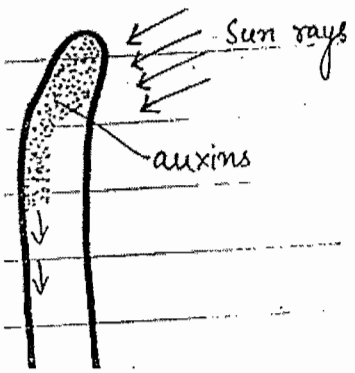


Figure 10: Effect of unilateral light on Auxin transport

Phototropism

Plant shoots display positive phototropism: when illuminated from one direction, the shoot proceeds to grow in that direction.

Proposed Mechanism:

- The direction of light is detected at the tip of the shoot.

- Blue light is most effective.
- It is absorbed by a flavoprotein called phototropin. Two flavoproteins, phototropins 1 and 2, are the photoreceptors for the blue-light signaling pathway Flavoproteins contain flavin as a prosthetic group.
- Auxin moves from the tip down.
- An Auxin transporter — one of the PIN proteins — is inserted in the plasma membrane at the lateral face of cells of the shoot which is not directly illuminated.
- Auxin is pumped out of these efflux transporters and accumulates in the cells on the shaded side.
- This stimulates elongation of the cells on the shady side causing the shoot to bend toward the light.

Gravitropism

Gravitropism is a plant growth response to gravity.

- Plant shoots display negative gravitropism: when placed on its side, a plant shoot will grow up
- Roots display positive gravitropism: they grow down.

Possible Mechanism of Gravitropism in Roots

- When a root is placed on its side, Statoliths (organelles containing starch grains) settle by gravity to the bottom of cells in the root tip.
- This causes PIN proteins to redistribute to the underside of the cell where they pump Auxin out of the cell; that is, they are efflux transporters.
- The Auxin then accumulates along the underside of the root.
- This INHIBITS root cell elongation. So the cells at the top surface of the root elongate, causing the root to grow down.

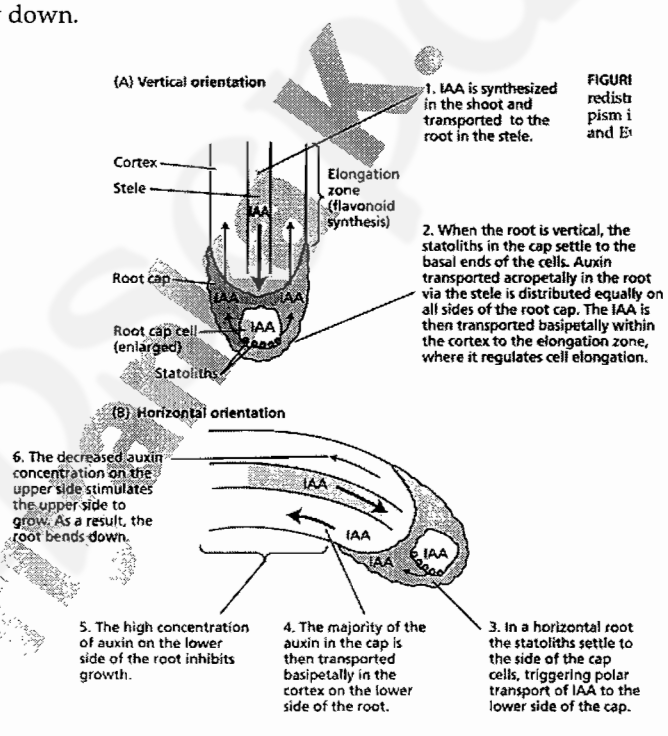


Figure 11: Proposed model for the redistribution of Auxin during gravitropism in maize roots.

Apical Dominance

Growth of the shoot apex (terminal shoot) usually inhibits the development of the lateral buds on the stem beneath. This phenomenon is called apical dominance.

If the terminal shoot of a plant is removed, the inhibition is lifted, and lateral buds begin growth. Gardeners exploit this principle by pruning the terminal shoot of ornamental shrubs, etc. The release of apical dominance enables lateral branches to develop and the plant becomes bushier.

The exact mechanism by which Auxin helps in establishing apical dominance is still an area of study by plant physiologists. However, the earliest model to describe the phenomenon, "the direct inhibition model" (Proposed by Thinmann & Skoog) now stands rejected, since most of the research findings indicate against the basic postulates of this model.

Recently, Pilate et. al. (1989) have found out that the phenomenon of apical dominance is also dependent on Cytokinin, apart from Auxins. The role played by Auxins is that of a mediator. According to this model, Auxins present in the apical bud increase the cell membrane permeability for Cytokinins - thus converting the shoot apex into cytokinin sink. Cytokinin, when present with Auxins, results into both rapid cell-divisions and also cell enlargement. Since the axillary buds do not have active Auxin activity, they also remain devoid of Cytokinins and hence show suppression of growth.

Fruit Development

Pollination of the flowers of angiosperms initiates the formation of seeds. As the seeds mature, they release Auxin to the surrounding flower parts, which develop into the fruit that covers the seeds.

Some commercial growers deliberately initiate fruit development by applying Auxin to the flowers. Not only does this ensure that all the flowers will "set" fruit, but it also maximizes the likelihood that all the fruits will be ready for harvest at the same time.

Abscission

Auxin also plays a role in the abscission of leaves and fruits. Young leaves and fruits produce Auxin and so long as they do so, they remain attached to the stem. When the level of Auxin declines, a special layer of cells – the abscission layer – forms at the base of the petiole or fruit stalk. Soon the petiole or fruit stalk breaks free at this point and the leaf or fruit falls to the ground.

Fruit growers often apply Auxin sprays to cut down the loss of fruit from premature dropping.

Root Initiation and Development

Although elongation of primary root is inhibited by Auxin concentration greater than 10-8M, initiation of lateral (branch) and adventitious roots is stimulated by high Auxin levels. In horticulture, the stimulatory effect of Auxin on the formation of adventitious roots has been very useful for vegetative propagation of plants by cuttings. Rooting is greatly enhanced if the cut surface of the cutting is dipped in Auxin solution or coated by commercial a rooting powder (which is generally NAA + Talcum powder).

On the basis of studies done on various alf mutants in Arabidopsis a model has been proposed which explains Auxin action in rooting promotion (Celenza et. al. 1995). According to this model, Auxin stimulates the root pericycle layer cells to divide and give rise to lateral roots.

Vascular Differentiation

New vascular tissues differentiate directly below developing buds and young growing leaves, and removal of the young leaves prevents vascular differentiation (Aloni 1995).

The relative amounts of xylem and phloem formed are regulated by the auxin concentration: High auxin concentrations induce the differentiation of xylem and phloem, but only phloem differentiates at low auxin concentrations.

Similarly, experiments on stem tissues have shown that low auxin concentrations induce phloem differentiation, whereas higher IAA levels induce xylem (Aloni 1995).

The regeneration of vascular tissue following wounding is also controlled by auxin produced by the young leaf directly above the wound site

Applications of Auxins

The uses of synthetic auxins in horticulture can be traced directly to the natural roles of IAA in the plant. In general, compounds such as a-naphthalene acetic acid (NAA) are used because they resemble IAA in action but are resistant to degradation by plant enzymes. Auxins are used for a variety of agricultural purposes, including:

- Promotion of rooting of cuttings (e.g., Rootone). The base of the cutting is dipped in a powder containing NAA or indolebutyric acid (IBA) prior to planting.
- Induction of flowering in pineapple (actually caused by the auxin-induced production of ethylene). NAA is generally employed as the auxin.
- Increased fruit set and induction of the pericarp in the absence of fertilization.
- Prevention of preharvest fruit drop.
- Auxin type herbicides (e.g., 2-4-D).

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Plant Hormones – II: Gibberellins

The Gibberellins are the second major class of growth hormones in plants, characterized by their ability to induce dramatic stem elongation in intact plants. They have been found in all the groups of plants, Algae, Fungi, Bryophytes, Pteridophytes, Gymnosperms and Angiosperms.

The Gibberellins are a large group of related compounds. By the end of 2008, **a total of 136 Gibberellins** have been identified in plants. Gibberellins are defined by their similar chemical structure rather than by their biological activity as applied in Auxins.

Discovery

Though Gibberellins became known to American and British scientists in the 1950s, they had been discovered in 1920s by Japanese scientists working on a fungal disease called *bakanae* or the "foolish seedling" that made the rice plants grow excessively tall but eliminated seed production. The diseased plants could not support themselves and died from combined weakness and parasitic attack.

The tallness of these plants was due a chemical secreted by the infecting fungus *Gibberella fujikuroi* [now identified as the asexual stage of *Fusarium moliniforme*]. This chemical was isolated from filtrates of the cultured fungus and called Gibberellin after the name of the fungus.

Gibberellin A_1 was the first Gibberellin to be isolated from a higher plant [in 1958 by J. McMillan]. Most of the Gibberellins in higher plants have been isolated from immature seeds. They contain a very high concentration of Gibberellins.

Chemistry & localization within the plant

Gibberellins constitute a large family of diterpene acids. All of them are called Gibberellic Acid with a different number to distinguish them [such as GA_1, GA_4, and GA_{29} etc.].

Their structure is based on the **ent-gibberellane** skeleton (Figure 1). The total number of C atoms is either 19 or 20 grouped in 4 or 5 ring structure. In almost all C-19 GAs, the carboxylic acid at carbon 19 bonds to carbon 10 and forms a lactone bridge. There are two other variations in the basic structure,

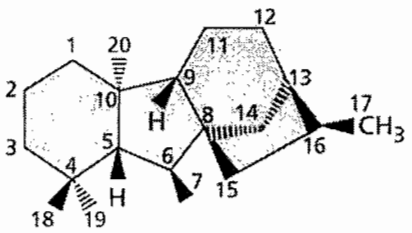
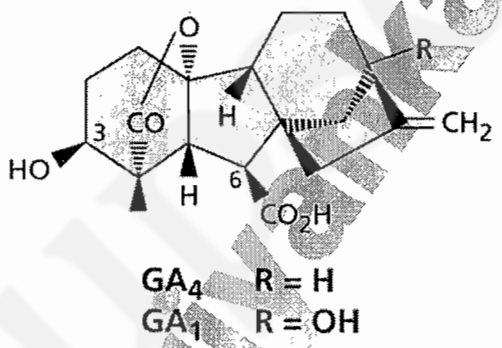
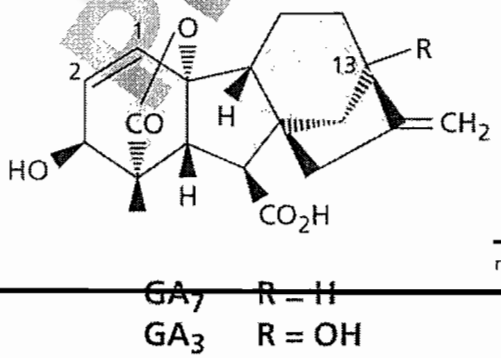


Figure 1: The ent-gibberellane skeleton

1. in the oxidation state of carbon 20 in C-20 GAs and the number; and
2. the position of hydroxyl groups on the molecule.

Despite the presence of a large number of Gibberellins found in plants, genetic analyses have demonstrated that only a few are biologically active as hormones. All the others serve as



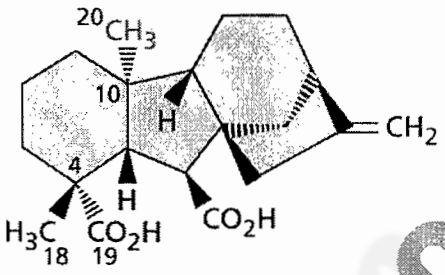


precursors or represent inactivated forms. The most biologically active GAs are GA_1, GA_3, GA_4, and GA_7 (Figure 2). These GAs, all of which have intrinsic or inherent stem growth-promoting activity, are C_{19}-GAs. They all possess a 4,10-lactone, a carboxylic acid (-COOH) at C-6, a hydroxyl group at C-3 in β-orientation, and do *not* have a hydroxyl group at C-2 in β-orientation. GA_{29} is an example of inactive Gibberellin.

Gibberellins that contain all 20 carbon atoms are referred to as **C_{20}-GAs.** The first-formed GA in plants is GA_{12}, a C_{20}-GA whose structure is shown below (Figure 3). The other GAs have only 19 carbons because they have lost C-by metabolism. These are referred to as **C_{19}-GAs**, and the structure of one of them, GA_9, is shown below in Figure 3.

Gibberellins are synthesized by a branch of **terpenoid pathway**. The immediate precursor to Gibberellin structure is GA_{12} aldehyde that is derived from an *ent*-kaurene ring. The GA_{12} aldehyde can be converted into all other Gibberellins, depending on the needs of the plant. Gibberellin biosynthesis is strongly influenced by two environmental factors:

1. Photoperiod
2. Temperature

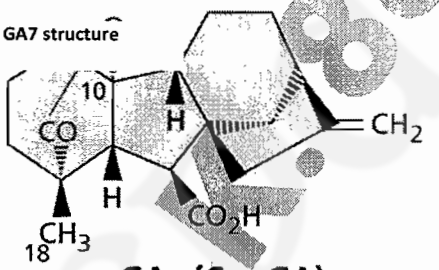


GA_{12} (C_{20}-GA)

Figure 12: GA 1, GA4, GA3 & GA7 structure

Moreover, the biosynthesis is also regulated by a feedback control.

The most active sites of Gibberellin biosynthesis in higher plants are immature seeds and young leaves. To some extent, roots also contribute.



GA_9 (C_{19}-GA)

In a mature plant, Gibberellins are found in high concentration in growing buds, leaves, and upper internodes. The active Gibberellin hormones are not translocated across a long distance. However, Gibberellin precursors, especially GA_{12} aldehyde, travel long distances through phloem translocation. Active Gibberellins are believed to be synthesized in the target tissues [**Frydmann**, 2001].

Figure 3: The structures of GA_{12} and GA_9.

Cellular mechanism of action

GA action in promotion of barley seed germination is well understood at cellular level. In this process, GA acts through cell surface located G-protein coupled receptors (GPCRs) as shown below.

1. GA_1 from the embryo first binds to a cell surface receptor.

2. The cell surface GA receptor complex interacts with a heterotrimeric G-protein, initiating two separate signal transduction chains.

3. A calcium-independent pathway, involving cGMP, results in the activation of a signaling intermediate.

4. The activated signaling intermediate binds to DELLA repressor proteins in the nucleus.

5. The DELLA repressors are degraded when bound to the GA signal.

6. The inactivation of the DELLA repressors allows the expression of the *MYB* gene, as well as other genes, to proceed through transcription, processing, and translation.

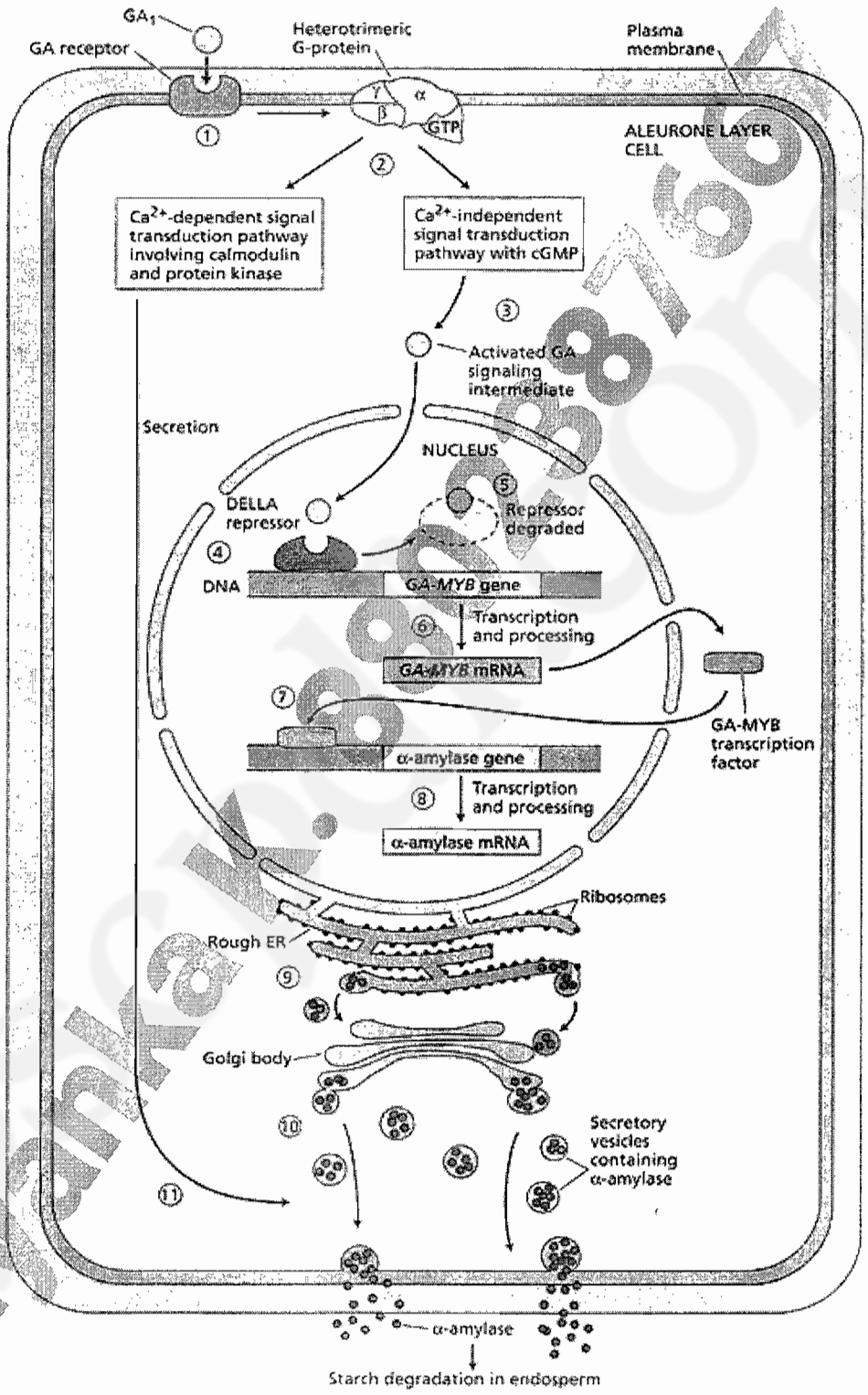
7. The newly synthesized MYB protein then enters the nucleus and binds to the promoter genes for α-amylase and other hydrolytic enzymes.

8. Transcription of α-amylase and other hydrolytic genes is activated.

9. α-Amylase and other hydrolases are synthesized on the rough ER.

10. Proteins are secreted via the Golgi.

11. The secretory pathway requires GA stimulation via a calcium–calmodulin-dependent signal transduction pathway.



Physiological Effects

Stem elongation

Internodal elongation is one of the best-characterized physiological effects of Gibberellins. Gibberellins can induce significant stem elongation in dwarfs or biennials in rosette stage. GA_1 is the primary Gibberellin responsible for stem elongation in most of the angiosperms.

It is now known that genetically dwarf plants [including those used by John Mendel in his breeding experiments] are deficient in Gibberellins. Exogenous Gibberellin application can make a dwarf plant overcome its genetic dwarfness, as seen in field experiments with dwarf varieties of maize, wheat, and pea. Genetically tall plants are now known to contain the ***Le* allele** of the gene that regulates the levels of active Gibberellins, while the dwarf plants have ***le* allele** of the same gene that results into a small amount of active Gibberellins in the plant.

Gibberellins also help a plant, especially biennials, in overcoming physiological dwarfness that is usually due to dependence on specific photoperiods or/and on a specific ambient temperature. For this reason, Gibberellins are also called **Bolting hormones.**

Gibberellins are capable of inducing stem elongation only in intact plants rather than in cut stem sections. This is one major difference between Auxin and Gibberellin action. The target of GA action is the intercalary meristem of stems. Roots do not show any response to Gibberellin application.

The exact combination of Gibberellins that can cause stem elongation varies from one plant to another. For example, rice and maize plants respond very well to exogenous application of GA_1+GA_3 while for the members of the family Pinaceae a combination of GA_4 and GA_7 has been found to be effective.

Regulation of transition from juvenile stage to adult phase

Gibberellins are capable of inducing a plant to make a transition between the juvenile stage and adult phase in both the directions. In *Hedera helix* application of GA_3 can induce the transition of an adult plant into the juvenile stage. An opposite effect, i.e. from juvenile to adult, is seen when GA_4+GA_7 is applied to conifer species.

This transition regulation mechanism is not well understood. As seen above, this effect varies with the plant chosen for experiment.

Floral initiation and sex determination

GA can substitute the long day or cold temperatures requirements for flowering in many plants, especially in biennials at rosette stage. GA_1 is usually combined to GA_3 and GA_4 to be used a flowering-inducing hormone. The exogenous application of GA induces a vegetative bud to differentiate into a floral bud. This transition and its molecular basis are not very clearly understood, but a direct correlation of floral initiation with GA application is well characterized through a number of field studies.

Sex determination in angiosperms is both genetically and environment regulated. Environmental regulation is correlated with Gibberellins.

In maize, short day and cool nights raise the levels of endogenous GA. This results into enhanced production of pistillate [female] flowers. The requirement of short day and cool nights for production of pistillate flowers can be substituted by exogenous application of GA.

In cucumber and spinach, production of staminate flowers is linked to increased levels of endogenous GA.

The effect in these plants can be artificially induced by exogenous application of GA.

Promotion of fruit set

Applications of Gibberellins can cause fruit set (the start of fruit growth following pollination) and growth of some fruit, especially in cases where Auxin may have no effect. Stimulation of fruit set by Gibberellin has been observed in apple *(Malus sylvestris)*.

Promotion of Seed Germination

Seed germination may require GAs for one of four possible steps:

1. The activation of vegetative growth of the embryo,
2. The weakening of a growth-constraining endosperm layer surrounding the embryo, and
3. The mobilization of stored food reserves of the endosperm.
4. For overcoming requirement of light or cold to induce germination in many wild plants

Gibberellin application stimulates the production of hydrolases, notably α-amylase, by the aleurone layers of germinating cereal grains. This allows rapid mobilization of stored food reserves of the endosperm for the use by growing embryo.

Commercial Applications

The major uses of Gibberellins (GA_3 unless noted otherwise) are in the management of fruit crops, the malting of barley, and the extension of sugarcane, with a resulting increase in sugar yield In some crops, a reduction in height is desirable, and this can be accomplished by the use of Gibberellin synthesis inhibitors.

FRUIT PRODUCTION: Gibberellins are used increase the stalk length of seedless grapes. Because of the shortness of the individual fruit stalks, bunches of seedless grapes are too compact and the growth of the berries is restricted. Gibberellin stimulates the stalks to grow longer, thereby allowing the grapes to grow larger because they have more room to do so.

A mixture of benzyl adenine (a cytokinin) and GA_4 + GA_7 can cause apple fruit to elongate and is used to improve the shape of apples under certain conditions. Although this treatment does not affect yield or taste, it is considered commercially desirable.

In citrus fruits, Gibberellins delay senescence; thus, the fruits can be left on the tree longer to extend the market period.

MALTING OF BARLEY: Malting is the first step in the brewing process. During malting, barley seeds *(Hordeum vulgare)* are allowed to germinate at temperatures that maximize the production of hydrolytic enzymes by the aleurone layer. Gibberellin is sometimes used to speed up the malting process.

INCREASING SUGARCANE YIELDS: Sugarcane is one of relatively few plants that store their carbohydrate as sugar (sucrose) instead of starch. The sucrose is stored in the central vacuoles of the internode

parenchyma cells. Spraying the crop with Gibberellin can increase the yield of raw cane by up to 20 tons per acre and the sugar yield by 2 tons per acre. This increase is a result of the stimulation of internode elongation during the winter season.

USES IN PLANT BREEDING: The long juvenility period in conifers can be detrimental to a breeding program by preventing the reproduction of desirable trees for many years. Spraying with GA_4 + GA_7 can considerably reduce the time to seed production by inducing cone formation on very young trees. In addition, the promotion of male flowers in cucurbits and stimulation of bolting in biennial vegetables such as beet and cabbage are valuable effects of Gibberellins that are occasionally used commercially in seed production.

GIBBERELLIN SYNTHESIS INHIBITORS: Gibberellin biosynthesis inhibitors are sometimes used commercially to prevent too much elongation growth in certain plants. In floral crops, short, stocky plants such as lilies and chrysanthemums are desirable, and restrictions on elongation growth can be achieved by applications of Gibberellin synthesis inhibitors such as **Ancymidol** (known commercially as A-Rest) or **Paclobutrazole**.

Phytochromes

Phytochrome is a pigment protein complex in all plants, many algae and even in some bacteria. It is sensitive to the red and far-red light. Many plant morphogenetic responses are dependent on red light signaling received by phytochromes. These include flowering, germination of seeds, elongation of seedlings, maturation of leaves etc.

Introduction to Phytochromes

Phytochromes are dimeric pigment protein complexes associated with plant photomorphogenesis. They sense red and far red wave lengths of light and convert these environmental signals into plant morphogenetic processes. These morphogenetic processes include flowering, germination of seeds, elongation of seedlings, maturation of leaves etc.

Phytochromes are also found in many algae and bacteria.

They were discovered by Sterling Hendricks and Harry Borthwick of the USA during a period from the late 1940s to the early 1960s.

Structure of Phytochromes

All phytochromes are homodimeric pigment protein complexes. The protein part is called *apoprotein*. The pigment part is called *chromophore*. Together, these two parts make the phytochrome *holoprotein*.

There are two peptide subunits, each with molecular weight of 125 kD. Thus, the total molecular weight is about 250 kD. Each peptide subunit has a large domain (~ 70 kD) and a small domain (~55kD). The two domains are connected by a small, flexible *hinge* domain.

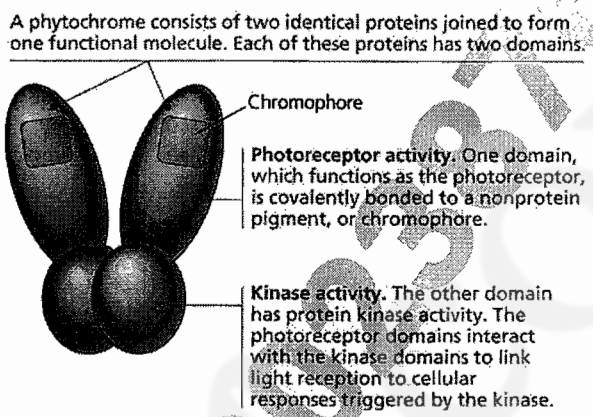


Figure 13: The schematic organization of phytochromes

The large subunit is covalently attached through a *thioether bond* to a bilin pigment. The pigment is blue in colour and it is sensitive to red and far red light wave lengths. The pigment is called *phytochromobilin*.

The schematic organization of phytochromes is shown in Figure 1.

Synthesis of Phytochromes

The phytochromes occur in the cytosol. They are never membrane integral. Most of the time, they migrate to the nucleus during active state.

The protein part of phytochromes is encoded by the nuclear genes. The phytochrome gene family is named *PHY*. Five such genes are recognized in *Arabidopsis thaliana* and the individual members are *PHYA, PHYB, PHYC, PHYD,* and *PHYE*.

In other plants, homologous genes are found but not necessarily all the five genes.

Phytochromobil in is synthesized inside plastids and is derived from 5-aminolevulinic acid. It leaks out of the plastid into the cytosol by a passive process. Assembly of the phytochrome apoprotein with its chromophore is autocatalytic; that is, it occurs spontaneously. The pigment binds to the protein by a *thioether bond* to a Cys- residue.

The Cellular Mechanism of Phytochrome Action

Phytochrome carries out light perception by its pigment part. The pigment, once activated, affects the conformation of the protein part. After this the protein part mediates the cellular functions.

The pigment, phytochromobilin exists in two forms.

a. *Cis-* form: In this form, it absorbs red light (660 – 680 nm) strongly. Therefore, when the protein is bound to the pigment in cis-form, the phytochrome is called

P_{Red} (P_R). In this form, the phytochrome is not active.

b. *Trans-* form: In this form, it absorbs far-red light (720 – 740 nm) strongly. Therefore, when the protein is bound to the pigment in cis-form, the phytochrome is called P_{FarRed} (P_{FR}). In this form, the phytochrome is physiologically active.

The P_R and P_{FR} forms are interconvertible, according to the following relation.

a. P_R strongly absorbs red light and gets converted into P_{FR}.

b. P_{FR} strongly absorbs far red light and gets converted back into P_R.

c. In some plants, P_{FR} goes through spontaneous dark conversion process and gets converted into P_R.

When Pr molecules are exposed to red light, most of them absorb it and are converted to Pfr, but some of the Pfr also absorbs the red light and is converted back to Pr because both Pr and Pfr absorb red light. Thus, even at red light saturation about 15% Pr forms can still be seen.

Similarly, the very small amount of far-red light is absorbed by Pr also. This makes it impossible to convert Pfr entirely to Pr by broad-spectrum far-red light. Instead, an equilibrium of 97% Pr and 3% Pfr is achieved. This equilibrium is termed the *photostationary equilibrium state*.

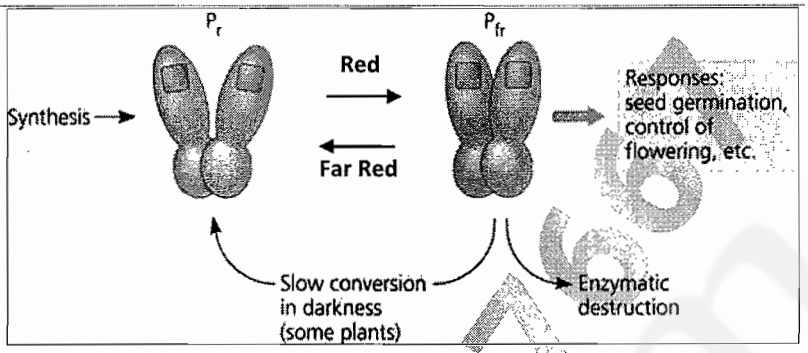


Figure 2: Phytochrome reversibility

As mentioned earlier, the **Pfr is the physiologically active form of Phytochrome.**

The light-induced cis to trans change in the pigment also affects the conformation of the polypeptide. It occurs both in the N-terminal chromophore-binding domain and in the C-terminal region of the protein.

The C-terminal domain of the phytochromes is a protein kinase domain. It is through this domain, phytochromes control cellular responses.

In most species, there are two different classes of phytochrome with distinct properties. These have been termed *Type I* and *Type II* phytochromes. The type I is far more abundant in most of the cells.

Phytochrome induced responses

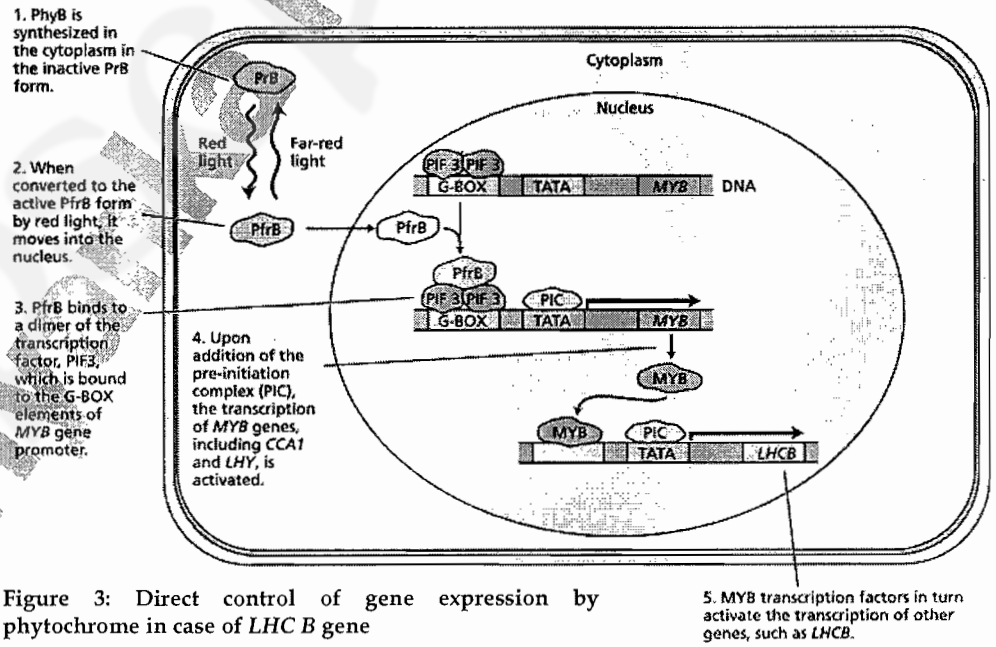


Figure 3: Direct control of gene expression by phytochrome in case of *LHC B* gene

are grouped into two types:

1. Rapid biochemical events
2. Slower morphological changes, including movements and growth.

The rapid responses are produced by the activation of cellular enzymes and membrane transporters by kinase activity of phytochromes. It is also known that in cytosol, phytochrome signaling involves several different mechanisms, including G-proteins and Ca^{2+} ion based signal relaying.

The slower responses are regulated at gene expression level. After being activated, some phytochromes expose their Nuclear Localization Signals (NLS) and move into the nucleus. Inside the nucleus, the kinase activity of Pfr stimulated transcription activation or repression complexes at specific gene promoters. This causes regulation of some genes and elicits cellular responses (Fig. 3). Phytochrome action stimulates genes for the small subunit of rubisco and the chlorophyll a/b–binding protein of the light-harvesting complex. Phytochrome also represses the transcription of various genes, including *PHYA*.

Phytochrome Responses

Phytochrome responses can be distinguished by the amount of light required. There are three types of phytochrome responses.

a. Very-Low-Fluence Responses (VLFRs)
b. Low-Fluence Responses (LFRs)
c. High-Irradiance Responses (HIRs).

VLFRs responses can be initiated by fluences as low as 0.0001 μmol m^{-2} and they saturate at about 0.05 μmol m^{-2}. For example, in dark-grown oat (*Avena*) seedlings, red light can stimulate the growth of the coleoptile and inhibit the growth of the mesocotyl (the elongated axis between the coleoptile and the root) at such low fluences. *Arabidopsis* seeds can be induced to germinate with red light in the range of 0.001 to 0.1 μmol m^{-2}.

The minute amount of light needed to induce VLFRs converts less than 0.02% of the total phytochrome to Pfr. Because the far-red light can convert only about 97% of the Pfr to Pr, far-red light cannot reverse VLFRs.

LFRs can be initiated at fluence above 1.0 μmol m^{-2}, and they are saturated at 1000 μmol m^{-2}. They include most of the red/far-red photoreversible responses (Figure 4).

HIRs require prolonged or continuous exposure to light of relatively high irradiance, and the response is proportional to the irradiance within a certain range.

The reason that these responses are called high-irradiance responses rather than high-fluence responses is that they are proportional to irradiance rather than to fluence. HIRs saturate at much higher fluences than LFRs—at least 100 times higher—and are not photoreversible.

Some HIRs include the following.

1. Synthesis of anthocyanin in various dicot seedings and in apple skin segments
2. Inhibition of hypocotyl elongation in mustard, lettuce, and petunia seedlings
3. Induction of flowering in henbane (*Hyoscyamus*)
4. Plumular hook opening in lettuce
5. Enlargement of cotyledons in mustard
6. Production of ethylene in sorghum

Importance of phytochromes

The discovery and characterization of bacterial phytochrome suggest that flowering-plant phytochrome evolved from a bacterial histidine kinase that participates in two-component signaling pathways.

Group	Genus	Stage of development	Effect of red light
Angiosperms	*Lactuca* (lettuce)	Seed	Promotes germination
	Avena (oat)	Seedling (etiolated)	Promotes de-etiolation (e.g., leaf unrolling)
	Sinapis (mustard)	Seedling	Promotes formation of leaf primordia, development of primary leaves, and production of anthocyanin
	Pisum (pea)	Adult	Inhibits internode elongation
	Xanthium (cocklebur)	Adult	Inhibits flowering (photoperiodic response)
Gymnosperms	*Pinus* (pine)	Seedling	Enhances rate of chlorophyll accumulation
Pteridophytes	*Onoclea* (sensitive fern)	Young gametophyte	Promotes growth
Bryophytes	*Polytrichum* (moss)	Germling	Promotes replication of plastids
Chlorophytes	*Mougeotia* (alga)	Mature gametophyte	Promotes orientation of chloroplasts to directional dim light

Figure 4: Typical photoreversible responses induced by phytochrome in a variety of higher and lower plants

Physiology of flowering

Introduction to Flowering & its basic control

Flowering is the developmental process of reproductive structures in the angiosperms. Early during this process a shoot vegetative meristem is converted into floral meristem, by differential gene expression. Subsequently, the floral meristem is elaborated in to a bud and finally into a flower.

Flowering occurs only at reproductive maturity. Before this, a plant is called juvenile.

Many species flower autonomously once they reach the reproductively mature stage. However, in most cases a reproductively mature plant flowers only when it is exposed to certain environmental signals. These environmental signals are called **florigenic signals** or **inductive factors of flowering** and the mainly include:

1. Photoperiod
2. Temperature

The other environmental, physiological and developmental factors which are necessary for flowering are:

1. Sufficient levels of biomass, mainly assessed by the number of leaves
2. Sufficiency of water in the soil
3. Sufficiency of minerals in the soil
4. Absence of temperature, wind and precipitation extremes
5. Absence of some seriously deleterious pathogenic effect

They are also known as the **permissive factors of flowering**.

Photoperiodism

Photoperiod means the relative duration of illumination on a twenty four hour scale. Any plant response dependent upon photoperiod is called a photoperiodic response and this phenomenon is called **photoperiodism**. A particular type of photoperiod is indicative of a certain time of the year. Thus, photoperiodic responses of plants ensure that certain developmental events (such as flowering) occur only at specific times of the year.

Photoperiodism was discovered by **W.W. Garner** and **H.A. Allard** (in early 1920s), while studying growth and flowering behaviour in Biloxi soybean (*Glycine max*) and in Maryland Mammoth variety of tobacco (*Nicotiana tabaccum*).

Operation of photoperiodism

Many plants flower only in a particular season. This season happens to be most suitable for floral development, pollination etc. and the season after this happens to favour seed and fruit dispersal.

Based on photoperiodic response of flowering, plants were traditionally grouped into following four categories:

1. Short day plants

2. Long day plants
3. Day neutral plants
4. Intermediate plants.

1. Short day plants flower readily only when the photoperiod is shorter than a critical period. Under longer (than the critical period) photoperiods, these species or varieties do not flower and remain in the vegetative state. This critical day length or the photoperiod depends upon the species. Some examples of short day plants are given below.

Monocots	Dicots
Winter rice (*Oryza sativa*)	Bryophyllum (*Bryophyllum pinnatum*)
	Chrysanthemum (*Chrysanthemum* spp.)
	Cocklebur (*Xanthium strumarium*)
	Hemp (*Cannabis sativa*)
	Kalanchoe (*Kalanchoe blossfeldiana*)
	Morning glory (*Ipomaea purpurea*)
	Strawberry (*Fragaria chiloensis*)
	Tobacco (*Nicotiana tabacum*)
	Violet (*Viola papilionacea*)

2. Long day plants flower readily only when the photoperiods longer than the critical photoperiod. The critical day length varies according to the species. For example, barley or wheat plants flower only when day length is more than 12 h, while spinach plants flower only when day length exceeds over 13 h. Some species such as *Agrostis palustris*, have a very long photoperiods, more than 16h. Some long day plants are listed below.

Monocots	Dicots
Barley (*Hordeum vulgare*)	Cabbage (*Brassica* spp.)
Bentgrass (*Agrostis palustris*)	Clover (*Trifolium pratense*)
Oats (*Avena sativa*)	Cone flower (*Rudbechkia bicolor*)
Orchard grass (*Dactylis glomerata*)	Dill (*Anethum gramveolens*)
Rye grass (*Lollium*)	Henbane (*Hyoscyamus niger*)
Timothy (*Phieum* spp.)	Petunia (*Petunia* sp.)
Wheat grass (*Agropyron smithii*)	Radish (*Raphanus sativus*)
Wheat (*Triticum vulgare*)	Spinach (*Spinacea oleracea*)

3. Day neutral plants flower readily over a wide range of day length from relatively short day length to continuous illumination.

Monocots	Dicots
Blue grass (*Poa annua*)	Azalea, coral bell (*Rhododendron* spp.)

Maize (*Zea mays*)

Balsam (*Impateins balsamina*)

Bean (*Phaseolus* spp.)

Cotton (*Gossypium hirsutum*)

Cucumber (*Cucumis sativus*)

Potato (*Solanum tuberosum*)

Tomato (*Solanum lycopersicum*)

4. Intermediate plants flower only under day lengths within a certain range and fail to flower under either longer or shorter photoperiods. In this category — some varieties of sugar cane, *Eupatorium hyssopifolium* and climbing hempweed (*Mikania scandens)*are included.

Perception of Photoperiod

Plants perceive photoperiods through their young leaves. In the leaves, phytochrome is the main light receptor that mediates the photoperiodic responses. It is located in leaf cell cytosol and sensitive to red and far red light in its alternate states.

In 2001, Chentao Linn showed that Cryptochromes, a class of Blue Light Receptors, also help in the process of photoperiodic responses to flowering.

Arabidopsis relies on at least nine photosensory receptors, including five phytochromes (PhyA-PhyE), two cryptochromes (cry1 and cry2), and two phototropins (phot1 and phot2), to regulate most of its light responses. Among these photoreceptors, phytochromes and cryptochromes are known to regulate flowering time. Studies indicate that cry2 and PhyA may act as major day-length sensors.

Plants perceive photoperiods by assessing the length of the night (Hammer and Bonner, 1946). Experimental studies establish that the lond day plants are actually short night plants and the short day plants are long-night plants (See the *Experiment Illustration in* Figure 1).

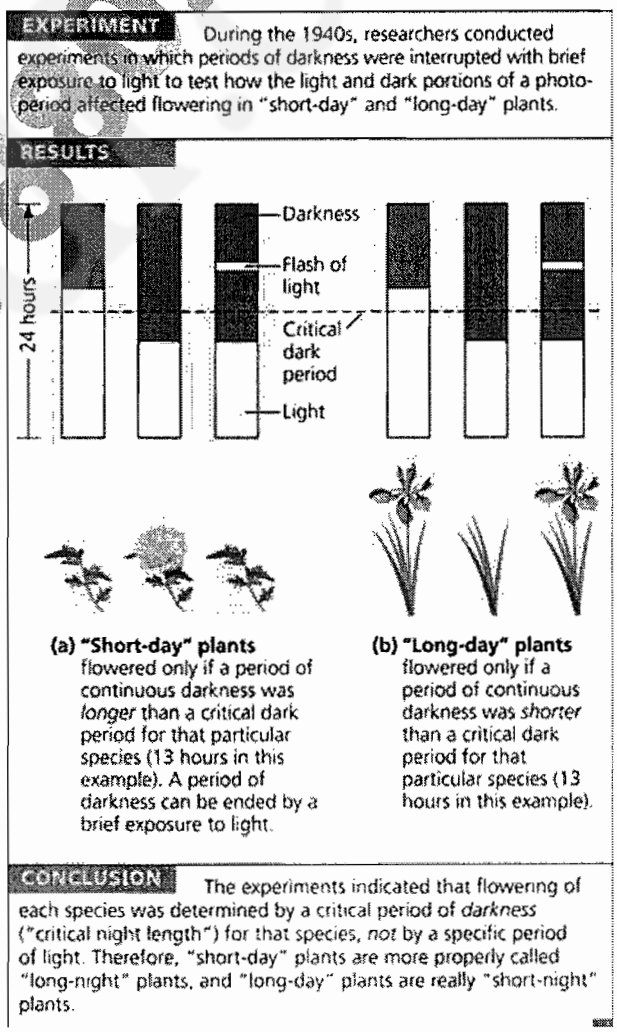


Figure 1: Experiment to show that plants perceive photo-periods by assessing the length of the night.

Mechanism of Photoperiodism

Photoperiod is a significant inducing factor of flowering in most plant species but the effect is very clear in the plants growing away from the equator. The plants which are native to the equatorial and tropical regions do not have strong photoperiodic induction of flowering. This is because the regions in the equatorial and tropical belts show by and large constant weather round the year and photoperiodic

sensing of the time of the year does not have much significance.

The photoperiodic induction of flowering occurs only in a reproductively mature plants. Such plants are also called *florally competent*. Juvenile plants in the same region donot respond to inductive photoperiods.

In many plant species, especially those native to the temperate regions, floral competence is brought about by a certain age, growth, number of leaves and a prolonged low temperature exposure. The dependence on low temperature exposure to become florally competent is known as *Vernalization*.

A florally competent plant receives the photoperiodic induction mainly through leaf cell located phytochromes (especially PhyA).

Required levels of PhyA builds up only during the long day periods. However, PhyA alone does not control the genes related to flowering. There is a *Clock Protein* also involved. This protein is encoded by a gene called *Constans* (CO) in *Arabibobsis*. Homologous genes are found in other plants also.

Photoperiodic induction is dependent upon the interaction between active PhyA and the Clock Protein. Subsequent to this interaction, the other genes associated with flowering are governed.

As currently understood, the long day and the short day plants use essentially same molecular machinery but to opposite effects.

Mechanism in Long-day Plant: *Arabidopsis*

Arabidopsis is a long-day plant. It uses the gene *CONSTANS* (*CO*) as the Clock Gene. *CONSTANS* encodes a zinc-finger transcription factor. The levels of CO mRNA rise and fall with a circadian rhythm. It has the following pattern.

1. CO mRNA level rise somewhat early in the morning
2. CO mRNA level declines during the middle part of the day.
3. CO mRNA level rises to a peak late in the afternoon.

Translation of CONSTANS mRNA mainly during morning and late in afternoon. The translation produces CO protein. However, the CO protein is quickly degraded in proteasomes during the morning and middle part of the day and also during the night. The morning degradation is triggered by light rich in 660 nm rays. It is mediated by phytochrome B (PhyB).

Under long day conditions, the degradation of CO protein ceases by late in the afternoon. This is because, by the afternoon sufficient levels of PhyA are activated. If CO protein is also present when active PhyA is available, then PhyA stabilizes CO protein. In this process Cry2 protein also has a role.

In short days, with darkness falling before the rise in CO protein, there is no PhyA available to stabilize CO protein.

CO protein is a transcription factor that activates a number of genes, including *FLOWERING LOCUS T* (*FT*). *FT* gene is needed to start the conversion of apical buds in flower buds.

As during the long days the CO protein accumulates in the cell, it activates the gene transcription of *FT*, especially in the phloem companion cells.

The FT transcript moves through the phloem to the shoot apex where the FT protein is synthesized (Huang *et al*; 2005). Some recent evidences, also indicate the movement of some FT protein through phloem from leaf to shoot apex (Blazquez *et al*; 2008).

The FT protein interacts with the transcription factor FD (already present in the shoot apex). The complex then activates key genes such as *AGL20* (also called *SOC1*) and *AP1* to start flower development. *SOC1* protein activates the gene *LEAFY* (*LFY*), which activates the genes responsible for meristem transition.

Mechanism in Short-day Plant: Rice

Ryousuke Hayama *et al* (2003) showed that the long day and the short day plants use essentially same molecular machinery but to opposite effects. In rice (*Oryza sativa*) – which is a a SD plant – they discovered the homologues of CO, and FT (found in *Arabidopsis*). These homologues are Hd1 and Hd3a respectively.

However, in rice these proteins act differently to control flowering.

In *A. thaliana*, under LD conditions, CO activates FT; but in rice, Hd1, which also accumulates under LD conditions, suppresses Hd3a expression, resulting in suppression of flowering under LD conditions. Thus, the basic gene network for the photoperiod control of flowering is conserved between *Arabidopsis* and rice, but the regulation of genes is reversed in the two species (Fig. 2).

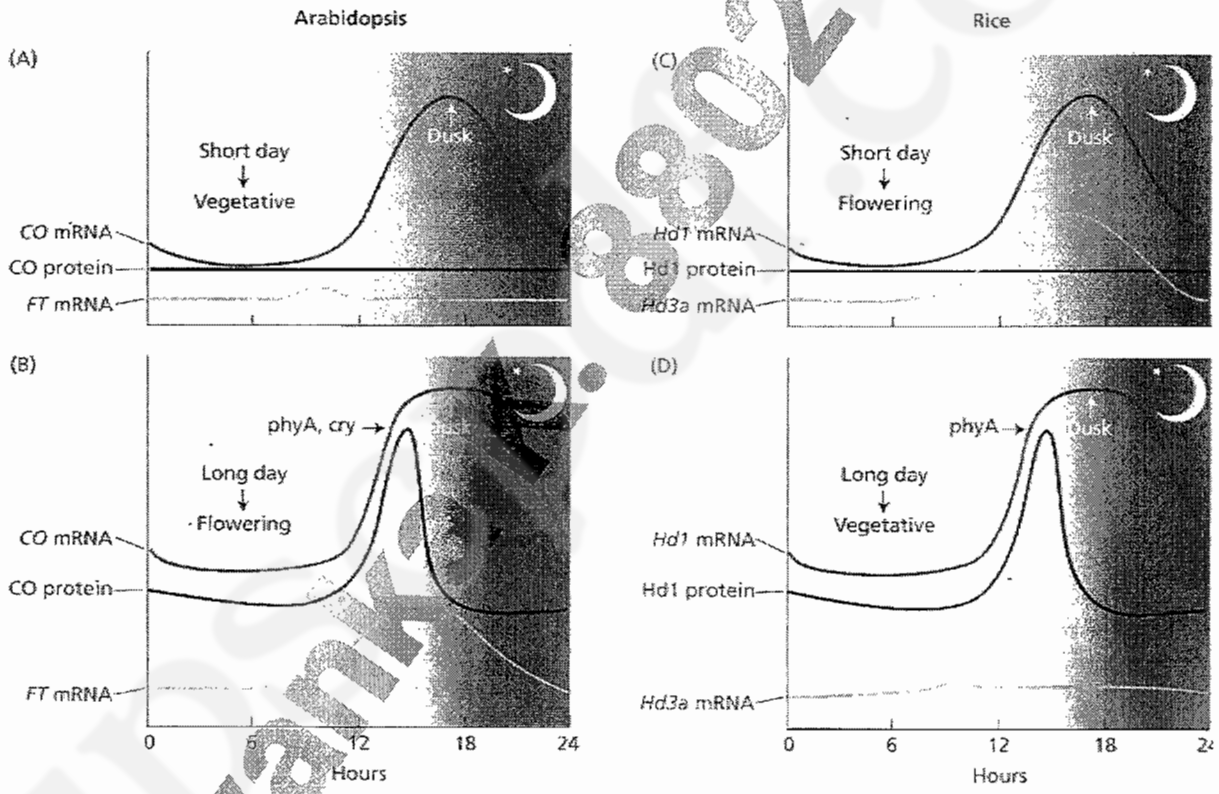


Figure 2: Presence of essentially same molecular machinery but using them to opposite effects by LDPs & SDPs.

Significance of Photoperiodism

1. With respect to flowering, photoperiodic control helps to:
   a. Promote cross-pollination, because synchronization of flowering time with a reliable environmental cue such as the photoperiod increases the chance of out-breeding and genetic recombination.
   b. Form the flowers at a favourable period of time
   c. Form seeds when the adverse conditions have arrived

d. Form seeds at a time when the dispersal agents are available

2. Photoperiodism explains the geographical distribution of plants that is why some plant species can be grown only in certain latitude. For example, Spinach, a long-day plant, cannot flower in the tropics because the days never get long enough (14 hours).

3. The floriculture (flower-growing) industry has applied photoperiodism knowledge to produce flowers out of season. *Chrysanthemum*, for instance, are short-day plants that normally bloom in autumn, but their blooming can be delated till early summers by punctuating each long night with a flash of light, thus turning one long night into two short nights.

Vernalization

Introduction to vernalization

In many species, especially those growing in the temperate parts of the world, temperature has a profound effect on flowering. Some plants do not flower even under the inductive photoperiod conditions, and flower only when a cold temperature treatment is given to the plant. This "**acquisition or acceleration of the ability to flower by a chilling treatment**" is termed as vernalization (Vernal = spring like).

The phenomenon was discovered by a Russian scientist T.D. Lysenko. He demonstrated that the winter varieties of wheat, rye and barley, could be planted in the spring to yield the crop at the same time as the summer varieties, if their moist seeds were subjected to an extended period of temperature near the freezing point.

The vernalization effect can also be visualized by examining the effect of chilling on flowering in winter rye, *Secale cereale*. It has two strains; spring and winter strain. The spring strain is an annual, flowering and fruiting in one growing season. The winter strain is a biennial; staying vegetative in the first growing season (fall and winter) and flowering and fruiting in the next season (summer). However, when the winter strain is vernalized, it can be planted in spring and it flowers late in summer.

Vernalization has an important role in plants of temperate region but very limited role in plants of equatorial and tropical belts. Nearly all biennial brassics show this effect.

Operation

Vernalization by itself does not induce flowering, but merely prepares the plant for flowering. Thus, it is factor providing floral competence to plants. The plants, after going through vernalising treatment, must also be exposed to inductive photoperiods.

Plants respond to chill treatment in the range of 0° C – 7° C, with the length of exposure being usually for a period of 2 to 8 weeks. After a prolonged treatment at cold temperature, the plant responds to a proper photoperiodic treatment and flowering is initiated.

This requirement of low temperature must be satisfied at any point in the juvenile stage. It varies from one plant to another. For example, wheat and rye respond best at soaked seed stage, while the brassics responds best at the rosette stage.

In the seedlings and in mature plants, *shoot apex* is the site of vernalization. The shoot which once receives the vernalization stimulus can translocate the stimulus to the other parts of the plant. Thus, once the tip is vernalized, the condition is transmitted to all other tissues formed subsequently, so that all other lateral tips are also vernalized.

In *Lunoria biennis*, however, it has been found that younger leaves are also capable of being vernalized, but older leaves, which have ceased growth, do not respond.

In plants, which can be vernalized at seed stage, plumule is the receptive tissue.

Physiological and biochemical changes during vernalization

Since freezing is not essential to bring about the changes during vernalization, it is physiological rather than purely physical process. This is further supported by the fact that cold treatment of rye grain is ineffective during anaerobic condition. In cultured plants and tissues, a supply of sugar also seems to be essential during vernalization.

It has been suggested that some kind of chemical is formed in the vernalized tip, which either induces florigen or/and makes the tissue receptive for the florigen action. **Melchers** and **Lang** suggested that a transmissible flowering stimulus is formed as a result of chilling. They called this stimulus **vernalin**. The 'vernalin' or any other such chemical substance has not been isolated so far.

Molecular events associated with vernalization is well studied in *Arabidopsis*.

It is now known that vernalization in *Arabidopsis* is controlled by the Flowering Locus C (FLC) gene. This gene plays an important role in suppressing flowering until after an extended period of coldness. *FLC* is on chromosome 5 and encodes a MADS-box protein, a transcriptional activator protein. It acts on other flowering repressing genes and ultimately represses flowering.

As long as *FLC* is active, flowering remains suppressed. The activity of *FLC* is controlled by another locus called *flowering locus D* (*FLD*). The key role of *FLD* is to stimulate flowering by repressing the action of *FLC*. *FLD* encodes a deacetylase enzyme, which removes acetyl groups from histone proteins in the chromatin surrounding the *FLC*. . The removal of acetyl groups from histones compacts chromatin structure and inhibits transcription.

The inhibition of transcription prevents *FLC* from being transcribed and removes its repression on flowering. In short, *FLD* stimulates flowering in *Arabidopsis* by deacetylating the chromatin that surrounds *FLC*, thereby removing its inhibitory effect on flowering (Figure 3).

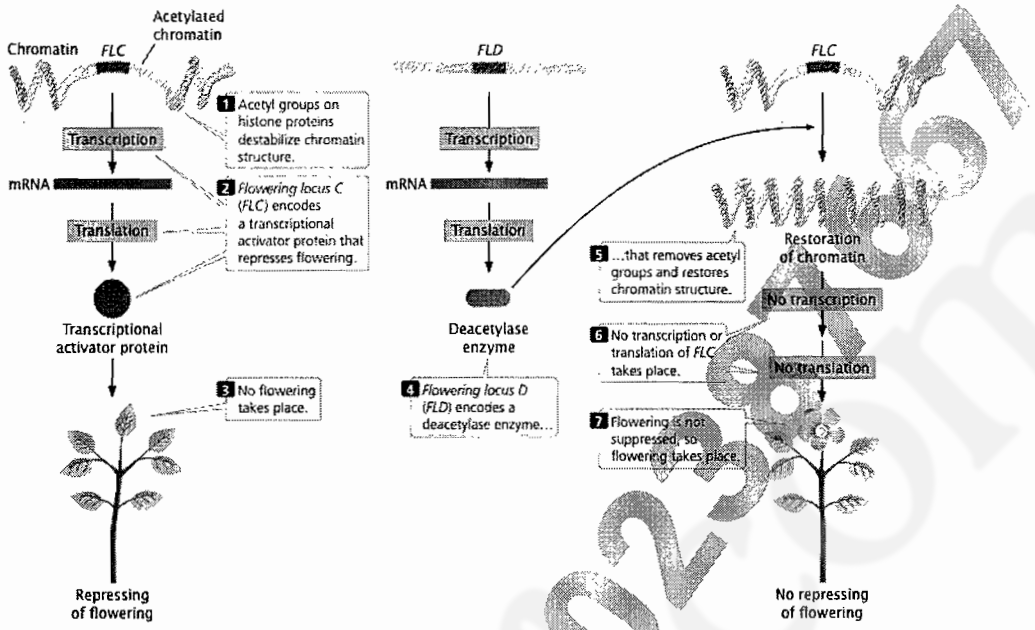


Figure 3: Removal of floral repression by vernalization in *Arabidopsis*

Several genes related to vernalization are now recognized.

They include:

1. *Arabidopsis*: *FRIGIDA.*
2. *Secale*: *VRN 3*
3. *Triticum aestivum*: *VRN 1, VRN 2*
4. *Triticum monococcum* (diploid wheat): *VRN-A^{m}1, VRN-A^{m}2*
5. *Hordeum vulgare*: *VRN-H1, VRN-H2*

Gene Activation during Flowering

Flowering involves sequential gene activation. Some of the genes are controlled by endogenous factors, while others are environmentally controlled. The Figure 4 summarizes our current understanding on gene activation during flowering and the influence of various factors (After Blazquez, 2008). Late during flowering, floral homoeotic genes are activated.

The floral meristem differentiates into four concentric groups of cells that form the four parts of the flower.

1. The cells in whorl 1 develop into a whorl of **sepals**. These form at the lowest level. Collectively they make up the **calyx**.

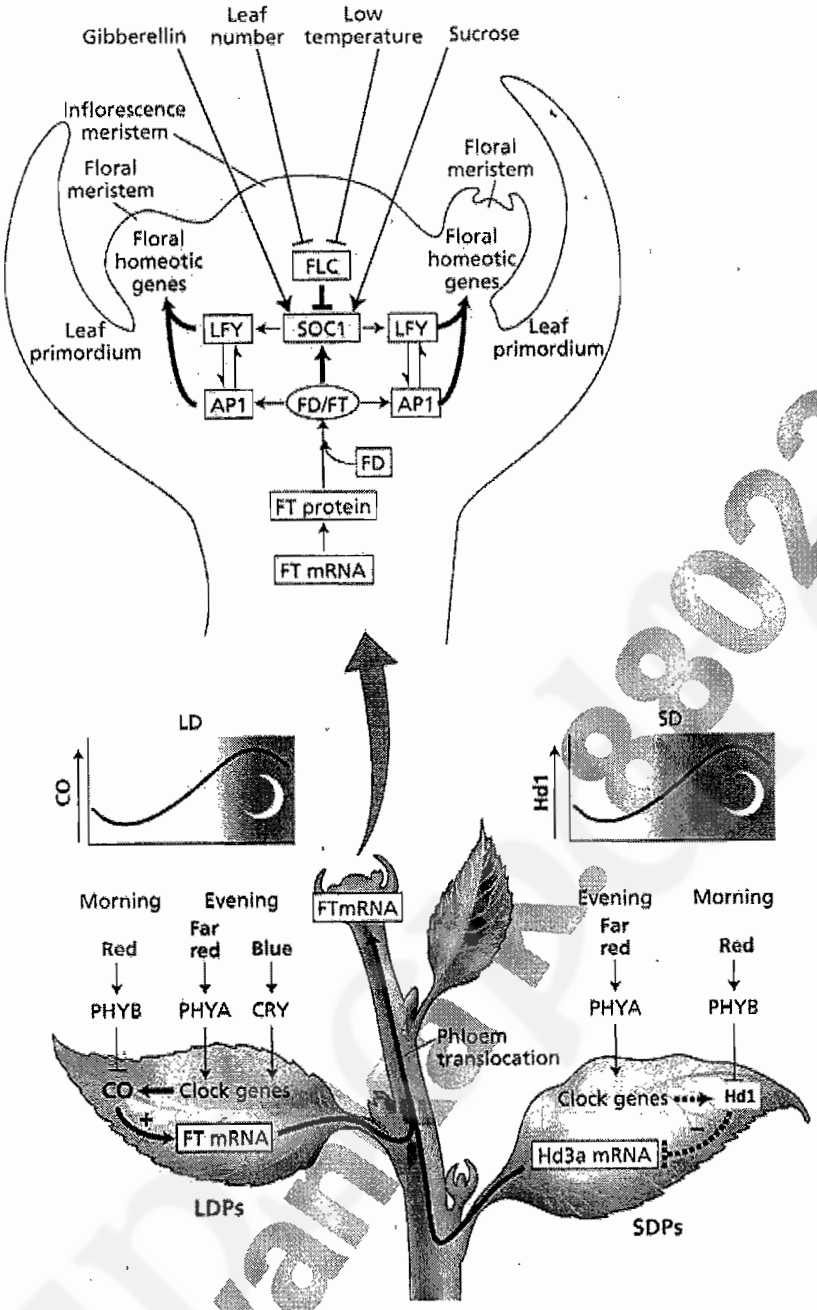


Figure 4: Gene activation during flowering

2. Whorl **2** forms above the calyx, forming the **petals**. Collectively these make up the **corolla** of the flower.
3. Whorl **3** develops into the **stamens**, the **male** reproductive organs.
4. The innermost whorl, 4, forms **carpels**, the female reproductive organs. Carpels often fuse to form a single structure, the **pistil**.

The ABC Model of Flower Development

Based on genetic analysis of mutants – especially those found in the dicots *Arabidopsis thaliana* and in the snapdragon (*Antirrhinum*), the ABC model of flowering was proposed by E. Coen and Elliot Meyerowitz in 1991.

This model postulates a group of genes that encode the transcription factors to form various floral whorls. These genes act as "master switches" and fall into 3 groups: A, B, and C. According to this model, the rules of floral organ formation are as follows.

1. Cells in which only **A genes** are expressed develop into **sepals**. This occurs at the lowest level of the floral meristem.
2. Cells in which both **A and B genes** are expressed develop into **petals**. This occurs at the next higher level.
3. Expression of **B and C genes** turns on the developmental program to form **stamens**.
4. Expression of **C genes** alone turns on the development of **carpels** in the innermost band of cells.

Thus, formation of a flower requires a **cascade of sequential gene activity** that gradually converts

Examples of A, B, and C group genes involved in flowering. These have been identified in *Arabidopsis thaliana*.	
A group	*APETALA1* (*AP1*) and *APETALA2* (*AP2*)
B group	*APETALA3* (*AP3*) and *PISTILLATA* (*PI*)
C group	*AGAMOUS* (*AG*)

a mass of undifferentiated cells (the apical meristem) into the parts of a flower.

Plant Hormones – III: Ethylene

Ethylene is the only plant hormone which is a gas at ordinary temperature. **It is an important determinant of stress response and senescence** in most of the plants.

Discovery

There were several indirect evidences in early 20th century to suggest that an emanation from the coal gas could cause senescence in leaves, fruits and also growth abnormalities in some plant parts.

- Farmers reported that if coal was burnt to keep the storage area warm, the ripening of fruits like apple and bananas was accelerated. The same effect was not seen if electrical bulbs were used to warm the storage area.
- When coal gas was used for street illumination, it was noticed that the trees near the street lamps shed their leaves much more than the trees away from the street lamps.

In 1901, Dimitry Neljubov reported that the coal gas present in the labs caused malformations in pea seedlings. These malformations were called the **Triple Response** which included:

1. Reduced Stem Elongation
2. Swelling
3. Abnormal Horizontal growth

Existence of a gas which causes ripening of fruits was first observed in 1910 by H. H. Cousins. He observed that a gas emanated from oranges caused ripening of banana stored together during shipping. The nature of the gas was only established in 1934 by R. Gane and it was found to be Ethylene, an unsaturated hydrocarbon.

Occurrence

Ethylene is produced in many algae, cyanobacteria, fungi (especially from Ascomycota), eubacteria and higher plants –including mosses, ferns, gymnosperms and angiosperms. Hence, **Ethylene is a plant hormone of ancient evolutionary origin.**

Ethylene production can take place in all living tissues. However there are some specific sites of high Ethylene synthesis.

- More Ethylene is produced in meristematic tissues and nodal regions.
- Dormant buds of apple and ripening climacteric fruits have high synthesis of Ethylene.
- Plucking, cutting or injury of tissues increases Ethylene production.
- Fungus infected crop plants release Ethylene in the crop fields to signal other healthy plants. Following this signals, the healthy plants increase the synthesis of defense molecules.

Bioassay

Ethylene mainly causes reduction in stem elongation and acceleration of senescence in plants; hence these properties are used for bioassay of Ethylene.

1. **Reduction in elongation of stem:** Plants enclosed in a chamber containing 0.01 ppm Ethylene show reduction in stem elongation, while at 1.0 ppm, increase in horizontal growth and stem swelling occurs. Bioassay is done in etiolated pea seedlings, sunflower, tomato, potato, castor and buckwheat.
2. **Acceleration of senescence:** Ethylene accelerates senescence of leaves, flowers, petals, fruits. When leaves are enclosed in a chamber containing Ethylene, loss in chlorophyll content is found. Senescence of cotton cotyledons is also accelerated.

Biosynthesis

In higher plants **chief source of Ethylene biosynthesis is amino acid methionine**. The process involves three steps as shown in *Figure 1*.

First step involves conversion of methionine into S-Adenosyl methionine (SAM). Methionine is activated by ATP molecule and enzyme involved here is SAM synthetase.

Second step involves cleavage of SAM into **1-amino cycloporpane-1-carboxylic acid (ACC)** and 5′methyl thioadenosine. The reaction is catalysed by enzyme **ACC synthase**. ACC formation controls the production of Ethylene. This step is directly correlated with Ethylene biosynthesis. Factor which enhances/inhibits the activity of enzyme ACC synthase also enchances/inhibits Ethylene biosynthesis. Auxins, stress factors (e.g. wounding, chilling, water strees, flooding pathogens), fruit ripening, leaf and flower senescence enchance activity of this enzyme, thereby, Ethylene production is also increase. Chemincals like aminoethoxy vinyl glycine (AVG) and amino oxyacetic acid (AOA) inhibit Ethylene biosynthesis by inhibiting activity of enzyme ACC synthase.

Third step involves oxidation of ACC to form Ethylene, CO_2 and HCN. HCN is further converted to formic acid and ammonia. The enzyme involved here is **ACC oxidase** which requires Fe^{++} and ascorbate for its activity. This step requires light and O_2. Activity of this enzyme is promoted by ripening, wounding and inhibited by sulfhydryl inhibitors (e.g. Co^{++}, Cu^{++}, Zn^{++}). ACC oxidase is not rate limiting unlike ACC synthase.

Regeneration of methionine occurs to continue further synthesis of Ethylene by recycling 5′ methyl thioadenosine (produced by clevage of SAM) via "Yang Cycle".

Synthesis of Ethylene increases with increase in temperature upto 35^0 C and decrease with decrease in temperature (0-20^0C). Ethylene synthesis is inhibited by low O_2 concentration. At high O_2 concentration plants do not respond to Ethylene.

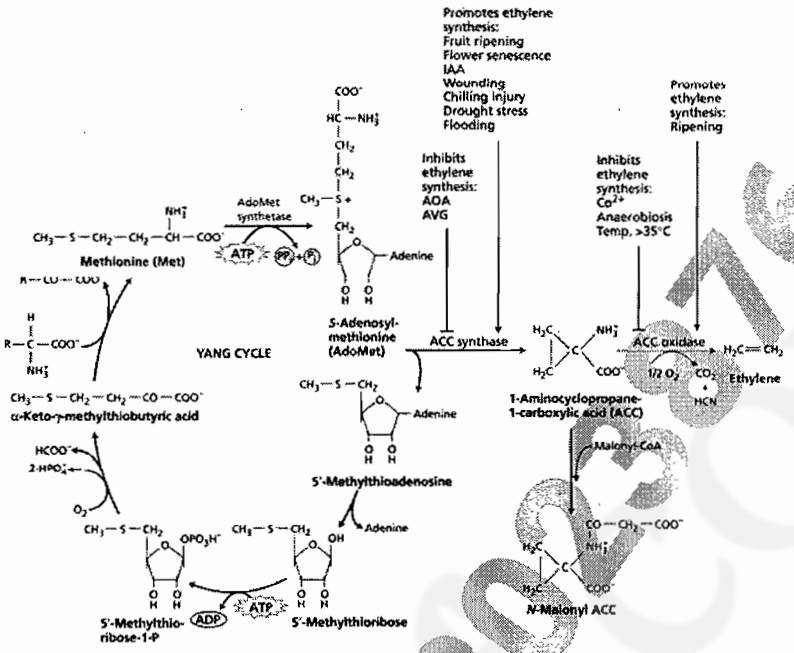


FIGURE 1: ETHYLENE SYNTHESIS PATHWAY

Physiological Effects

Ethylene is the **principal senescence hormone of angiosperms, mediating leaf, fruit and flower senescence**. It is also a **multifunctional stress signaling agent**.

Main responses of Ethylene are briefly explained below.

1. ***Fruit ripening.*** As fruits mature, the rate of ACC and Ethylene biosynthesis increases. Ethylene brings about most of the changes associated with fruit ripening. Such changes typically include softening due to the enzymatic breakdown of the cell walls, starch hydrolysis, sugar accumulation, and the disappearance of organic acids and phenolic compounds, including tannins. All fruits that ripen in response to Ethylene exhibit a characteristic respiratory rise before the ripening phase called a climacteric. Such fruits include: *Apple, Banana, Peach, Pear, Persimmon, Plum, Tomato* etc.
2. ***Leaf epinasty*** The downward curvature of leaves that occurs when the upper (adaxial) side of the petiole grows faster than the lower (abaxial) side is termed epinasty. Ethylene and high concentrations of auxin induce epinasty, and it has now been established that auxin acts indirectly by inducing Ethylene production.
3. A variety of stress conditions, such as salt stress or pathogen infection, increase Ethylene production and also induce epinasty. There is no known physiological function for the response. In tomato and other dicots, flooding (waterlogging) or anaerobic conditions around the roots enhances the synthesis of Ethylene in the shoot, leading to the epinastic response. Because these environmental stresses are sensed by the roots and the response is displayed by the shoots, a signal from the roots is transported to the shoots. This signal is ACC, the immediate precursor of Ethylene. ACC levels were found to be significantly higher in the xylem sap after flooding of tomato roots for 1 to 2 days.
4. ***Formation of Roots and Root Hairs*** Ethylene is capable of inducing adventitious root formation in leaves, stems, flower stems, and even other roots. Ethylene has also been shown to act as a positive

regulator of root hair formation in several species. This relationship has been best studied in *Arabidopsis*. In Ethylene-treated roots, extra hairs form in abnormal locations in the epidermis. Seedlings grown in the presence of Ethylene inhibitors (such as Ag^+), as well as Ethylene-insensitive mutants, display a reduction in root hair formation in response to Ethylene. These observations suggest that Ethylene acts as a positive regulator in the differentiation of root hairs. Ethylene also **stimulates lateral enlargement of roots** (e.g. radish) but not the elongation of root. Root elongation is inhibited by Ethylene.

5. ***Germination and sprouting*** Ethylene stimulates seed germination in barley and other cereals. It stimulates the action of hydrolytic enzymes in storage tissues. This also causes sprouting and germination in dormant potato tubers, dormant stolons, corms and rhizomes. The basis of this action is not well understood.
6. ***Effect on stem growth*** Ethylene inhibits internode elongation. It thus **inhibits the longitudinal growth of stem** but in turn it **causes lateral growth or swelling** of stem which leads to increase in girth of stem. The lateral swelling is due to cell enlargement and not due to cell division. Ethylene mediated inhibition on longitudinal growth is transitory, plants resume normal growth if Ethylene is removed. Ethylene mediated inhibition can also be overcome by CO_2 application. **High concentration of Ethylene in paddy in response to flooding** promote growth in height, which helps the plants in emerging out of submerged water.
7. ***Aerenchyma formation.*** Under extended flooding periods, Ethylene begins to cause selective and controlled cell death in the parenchymatous region, leading to the formation of aerenchyma. This is important for maintaining optimal aeration of the root tissue.
8. ***Effect on Flowering.*** Ethylene inhibits flowering in plants almost universally. However, pineapple, plumbago, cucurbits are exceptions. If floral buds have emerged, Ethylene would promote the blooming. Ethylene induces flowering in S.D. plants even in long day condition.
9. ***Sex Determination.*** Ethylene application reduced the male flower formation and **favour female flower formation**. Ethylene enhances pistillate flower formation in cucurbits. Ethylene application in vegetative body causes uniform female flowering. As a result, more fruits are produced by Ethylene treatment but is size of fruits is smaller. Examples are - cucumber, pumpkin, ridge gourd, melon etc. The role of Ethylene in **Sex Reversal is well understood in Papaya.** The feminizing influence of auxins is due promotion of Ethylene biosynthesis.
10. ***Abscission*** The shedding of leaves, fruits, flowers, and other plant organs is termed abscission. Abscission takes place in specific layers of cells, called abscission layers. Weakening of the cell walls at the abscission layer depends on cell wall-degrading enzymes such as cellulase and polygalacturonase. Ethylene appears to be the primary regulator of the abscission process.

A model of the hormonal control of leaf abscission describes the process in three distinct sequential phases (Reid 1995):

1. *Leaf maintenance phase*. Prior to the perception of any signal (internal or external) that initiates the abscission process, the leaf remains healthy and fully functional in the plant. A gradient of auxin from the blade to the stem maintains the abscission zone in a nonsensitive state.
2. *Shedding induction phase*. A reduction or reversal in the auxin gradient from the leaf, normally associated with leaf senescence, causes the abscission zone to become sensitive to ethylene.
3. *Shedding phase*. The sensitized cells of the abscission zone respond to low concentrations of endogenous ethylene by synthesizing and secreting cellulase and other cell wall-degrading enzymes, resulting in shedding.

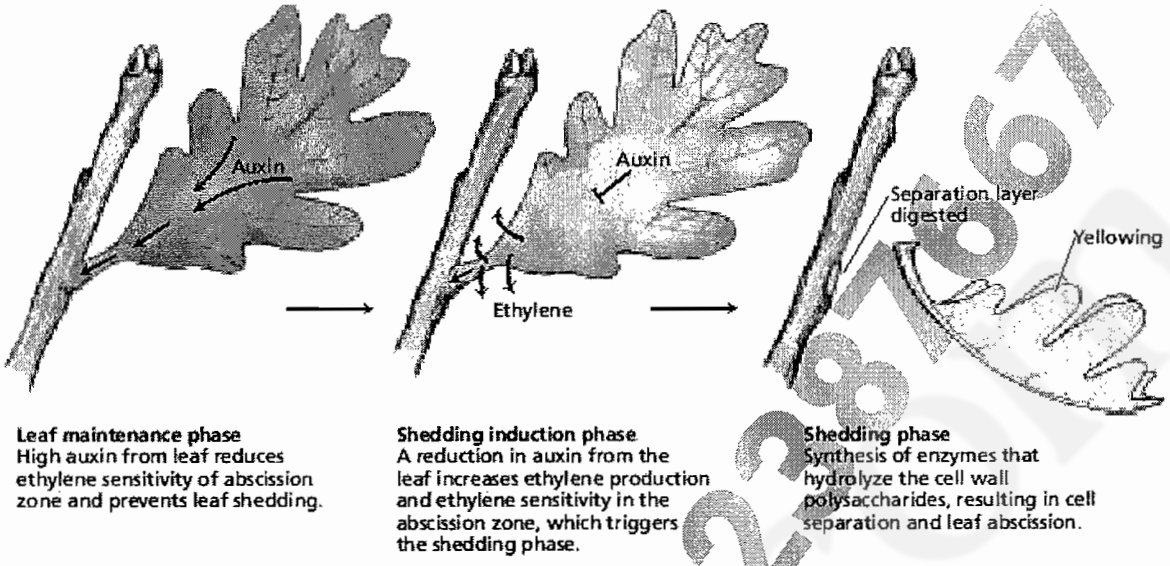


FIGURE 2: LEAF ABSCISSION PROCESS

Mechanism of Ethylene Action

Ethylene works on target cells using a **two component signaling pathway,** as shown below. Significantly, the receptors are located in the ER membrane and they get negatively regulated by Ethylene. The main receptors for Ethylene are ***ETR1, ETR2, ERS 1, ERS 2*** and ***EIN4***.

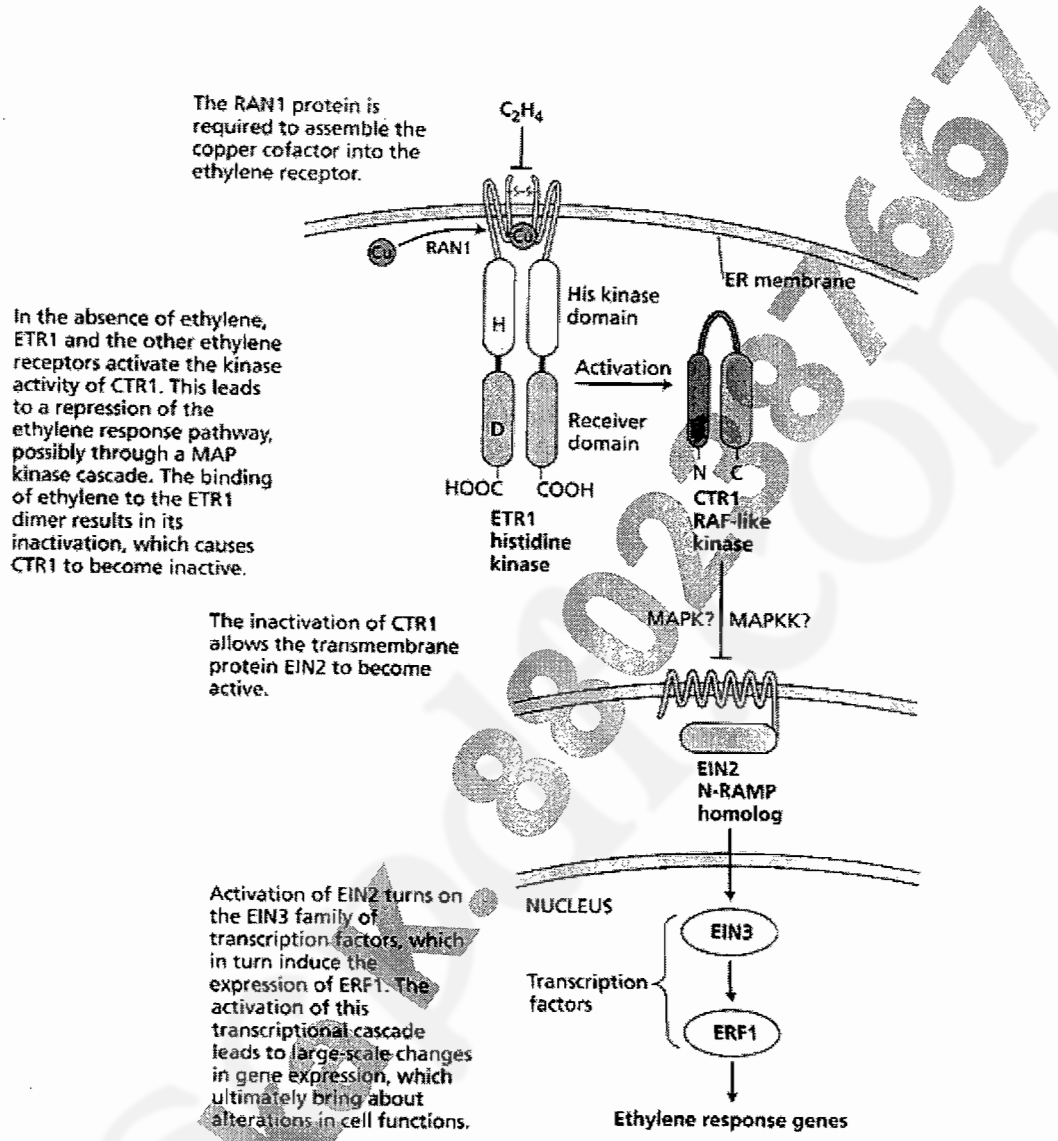


FIGURE 3: ETHYLENE SIGNALING PATHWAY

Commercial Uses

Ethylene has been used for synchronized flowering and fruit ripening for centuries. The main applications of ethylene are as follows.

1. Several chemicals which release Ethylene are used now-a- days for promotion of flowering in pine apple. Ethrel or Ethephon (2 chloroethyl phosphoric acid) is Ethylene releasing chemical. It rapidly breaks down in water to produce Ethylene. Other Ethylene releasing chemicals are- BOH, ethylpopyl phosphate, monoethyl sulphate.
2. In crop lands Auxins are applied to get certain desired effects of Ethylene, like ripening acceleration and defoliation of weeds.
3. Ethylene is also used for induction of feminity in cucurbits.

4. Ethylene is also used to increase the number of fruits in cucurbits.
5. Ethylene is widely used in fruit ripening, improvement of colour and flavour (i.e. qualities related to ripening).
6. Ethylene is *antagonist* to auxin that is used to curtail the tendency of lateral branching.
7. Ethylene is used to break bud dormancy in several overwintering plants like *Arachis hypogea*.

Ethylene synthesis or action inhibitors like low O_2 conc., high CO_2, low pressure [vacuum], low temperature, Ag^+ ions, AVG, trans-cyclooctane and 1-methylcyclopropane [MCP] are used to delay flower or fruit senescence in storage.

Transgenics with expression of an anti-sense version of ACC oxidase have been developed for tomato and petunia, where floral and fruit senescence have been delayed by several weeks.

Plant Hormone – IV: Cytokinins

What are Cytokinins?

Cytokinins or CKs are a class of plant hormones — with the chemical structure of N^6 substituted aminopurines — active in promoting cell division, cell growth, differentiation, senescence delay and other physiological processes. The CKs are:

- universal plant hormones, which
- act in a constitutively critical way.

So far, no member of plantaeviridae has been found to be a CK mutant. The only other phytohormone to show this behaviour is Auxin.

The principal CK in higher plants is **Zeatin**, that is *trans* – 6 – (4 – Hydroxy – 3 methylbut – 2 – enylamino) purine.

Discovery

- In 1913, Gottlieb Haberlandt discovered that a compound found in phloem had the ability to stimulate cell division in cultured plant cells.
- In 1941, Johannes van Overbeek discovered that the milky endosperm from coconut also had this ability. He also showed that various other plant species had different compounds which stimulated cell division.
- In 1954, the first cytokinin was isolated from autoclaved herring sperm by Miller and his associates.This compound was named **kinetin** (6 – furfuryl aminopurine) because of its ability to promote cytokinesis.
- Hall and deRopp reported that kinetin could be formed from DNA degradation products in 1955.
- Kinetin, however, could not be isolated from plants. The first plant derived cytokinin was isolated from corn in 1961 by Skoog and Miller. It was later called **zeatin**.
- Letham, in 1962 – 63, published a report on zeatin as a factor inducing cell division and later described its chemical properties.
- It is Skoog, Miller and Letham that are credited with the simultaneous discovery of zeatin.
- Since then, many more naturally occurring cytokinins have been isolated and characterized.

Distribution

Today there are more than 200 natural and synthetic cytokinins. Cytokinins have been found in

- All the vascular plants
- Nearly all the green algae and several Diatoms
- Mosses — where CK plays an essential role in protonema development
- Fungi — where CK plays an essential role in hypha development and host malformation development as in the infection by *Synchytrium*.
- Bacteria — such as *Agrobacterium* (for host exploitation) and *Corynebacterium fascians* that causes Witches Broom condition in *Abies balsamia*
- Insects
- Root knot nematodes — to exploit the host
- tRNA of many prokaryotes and eukaryotes as a hypermodified base.

Within a plant, Cytokinin concentrations are highest in meristematic regions and areas of continuous growth potential such as root apex, young leaves, developing fruits, and seeds.

Chemical Nature

There two kinds of cytokinins:

1. Adenin Cytokinins: Examples: kinetin, zeatin, benzyl adenine (same as 6-Benzylaminopurine).
2. Phenylurea Cytokinins (always artificial): Example: N, N'-diphenylurea, Thidiazuron

Although their chemical compositions differ, there is a structural correlation between adenine cytokinins and urea cytokinin.

Three most important and abundant naturally occurring CK in plants are:

1. *Trans* — Zeatine
2. N^6 isopentyl adenine
3. Dihydrozeatine

They are present as ribosides, ribotides or glycoside. However, studies based on CK receptor CRE1 show that the hormonally active form of CK is free base and not the conjugated forms. Perhaps, the conjugated forms play a role in long distance transport of CKs.

Widely used synthetic CKs include:

1. Benzyl aminopurine
2. N-N′ Diphenyl urea
3. Thidiazuron

Synthesis

Cytokinin is generally found in higher concentrations in meristematic regions and growing tissues. Cytokinin biosynthesis happens through the biochemical modification of adenine.

1. A product of the mevalonate pathway called isopentyl pyrophosphate is isomerized.
2. This isomer can then react with adenosine monophosphate with the aid of an enzyme called isopentenyl AMP synthase.
3. The result is isopentenyl adenosine-5'-phosphate (isopentenyl AMP).
4. This product can then be converted to isopentenyl adenosine by removal of the phosphate by a phosphatase and further converted to isopentenyl adenine by removal of the ribose group.
5. Isopentenyl adenine can be converted to the three major forms of naturally occurring cytokinins.

Transport

- Rapidly transported in xylem stream at the plant level in conjugated forms.
- Small amount may move through phloem
- At the cell level, transported by purine transporters
- Action is exerted mostly extracellularly and rarely intracellularly.
- Released by meristematic cells when they have enough minerals and water to support both themselves and any dependent cells

Actions

CKs are characterized at Bioassay level by:

1. Induction of cell division in Callus in the presence of Auxins
2. Promotion of bud or root formation when applied in a proper molar concentrations with Auxins
3. Delay Senescence of Leaves (Richmond – Lang Effect)
4. Expansion of Dicot Cotyledons

Important Physiological Effects

1. Cell division promotion — • by promoting D type cyclin production • by promoting the formation of *Cdc 25* type phosphatase • thus helping the activity of Auxin encoded CDC2 Kinase
2. Modification of Apical Dominance (Auxin enhances CK receptor formation)
3. Delay of Leaf Senescence (Richmond – Lang Effect)
4. Promotes Chlorophyll production and leaf unrolling
5. Promotes photosynthesis
6. Stimulates cell broadening leading to cotyledon and leaf expansion
7. Promotes shoot formation
8. Promotes the unloading of sugar from phloem
9. Participates in morphogenesis
10. Promotes moss protonema development
11. Directly induces GA/BS at high levels
12. Inhibits C4 Photosynthesis at low temp
13. Stimulates the rate of metabolism of cells in the shoot (that are not at their peak metabolism rates) in response to an increase in the levels minerals and water

Richmond Lang Effect

The delay of senescence of leaves and other organs of the plants by cytokinins is called Richmond - Lang effect. It was discovered by Richmond and Lang in 1957.

The manifestation of the effect

Richmond and Lang (1957) initially reported that exogenous cytokinins could delay senescence onset in detached leaves.

Most researchers who confirmed cytokinin-induced senescence delaying have used excised leaves in the dark. The physiological response of cytokinins *in vivo*, however, are often less pronounced and dramatic than *in vitro*. Therefore, the anti-senescent role of cytokinins generally has been significant to tissue cultures and post-harvest preservation of horticultural products.

If a young excised leaf is kept in water, it slowly changes its colour to yellow and dies. If such leaves are provided with cytokinin, the yellowing is significantly delayed.

The hormonal control of the Richmond-Lang effect

Richmond Lang effect is retardation of ageing due to cytokinins.

Although plant senescence is a complex syndrome of biochemical and physiological changes, the anti-senescent effect of cytokinins has generally been attributed to the following factors.

1. retarding chlorophyll loss

2. inhibiting protein degradation
3. preventing effluent carbohydrate translocation
4. mobilising metabolites

In 1984, Jidey *et. al.* suggested that this effect of cytokinin has been explained as due to the prevention of degradative catabolic processes by the way of repression activity of few hydrolysing enzymes like protease, RNAse, DNAse etc.

Furthermore, cytokinin facilitates the chlorophyll synthesis.

It also sustains the activity of carbon fixation, RNA synthesis and protein synthesis.

These inputs notwithstanding, the exact mechanism by which cytokinins prevent ageing and senescence is not yet known.

Cytokinin receptors

The cytokinin receptors have been identified and they are like the **two-component signalling system** common in bacteria

- characteristic in this system is a receptor with a histidine kinase domain
- when activated by binding the hormone, the kinase transfers a phosphate to an aspartate, then to a phosphotransfer protein
- the phosphotransfer protein then phosphorylates the response protein, which becomes active as a transcription factor or may affect some other cellular response to cytokinin
- there are multiple cytokinin receptors and many different response proteins, implying redundancy of function and/or specialization of response functions

Plant Hormones – V: Abscisic Acid

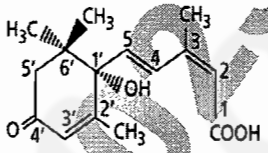
What is Abscisic Acid?

Plant growth and development are regulated by internal signals and by external environmental conditions. One important regulator that coordinates growth and development with responses to the environment is the 15 carbon sesquiterpenoid hormone Abscisic acid (ABA).

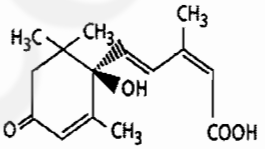
ABA plays important roles in many plant physiological processes including seed development, dormancy, germination, vegetative growth, and environmental stress responses under conditions like water stress and chill stress.

Chemistry of Abscisic Acid

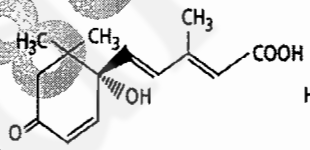
- Abscisic acid is a single compound unlike the auxins, gibberellins, and cytokinins.
- **The chemical name:** [S-(Z,E)]-5-(1-Hydroxy-2,6,6-trimethyl-4-oxo-2-cyclohexen-1-yl)-3-methyl- 2,4-pentanedienoic acid.
- **The chemical formula:** $C_{15}H_{20}O_4$
- The orientation of the carboxyl group at Carbon 2 determines the *cis* and *trans* form of ABA. **Naturally active ABA is always in the *cis*-isomeric form.**
- ABA has an asymmetric carbon at position 1′ of the ring, resulting into S and R (or + and –) enantiomers. For long term responses (such as seed dormancy), both the enantiomers are equally effective. However, for quicker responses, the S form is more active. The enantiomer R-ABA does not occur in nature, but constitutes 50% of commercially available ABA.



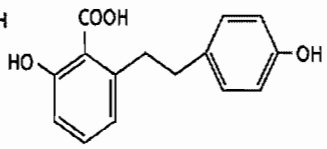
(*S*)-*cis*-ABA (naturally occurring active form)



(*R*)-*cis*-ABA (inactive in stomatal closure)



(*S*)-2-*trans*-ABA (inactive, but interconvertible with active [*cis*] form)



Lunularic acid (liverworts)

History of Abscisic Acid

- In 1963, Abscisic acid was first identified and characterized from cotton bolls by Frederick Addicott and his associates. They were studying compounds responsible for the abscission of fruits (cotton). Two compounds were isolated and called abscisin I and abscisin II. The name Abscisin was coined originally because it was thought to play a major role in abscission of fruits. Abscisin II is presently called Abscisic acid (ABA).
- The name **Abscisic acid** and abbreviation *ABA* were recommended by a panel to the 1967 International conference on Plant Growth Substances. The recommendation was accepted by the Conference and the term Abscisic acid is now in universal use.

Synthesis, Translocation and Degradation of Abscisic Acid

- In most well-watered plants, **ABA is predominantly synthesized in the mesophyll cytoplasm of mature, green leaves** but, because of intracellular pH gradients, ABA accumulates in the chloroplasts. At low pH,

ABA exists in the protonated form ABAH, which freely permeates most cell membranes. The dissociated form ABA is impermeant.

- **Under water stress conditions, the roots become the major site of ABA synthesis**.
- Abscisic acid is able to move quickly out of the leaves to other parts of the plant, especially sink tissues. Leaf to sink transport of ABA occurs through the phloem.
- Root to stem translocation of ABA during water stress conditions is through the xylem.
- **Synthesis:** Abscisic acid is a 15-cabron sesquiterpene, a number and arrangement of carbon atoms that suggest **ABA is derived from mevalonic acid**.

Physiological Actions of Abscisic Acid

In bioassays, ABA is characterized primarily by:

1. Inhibition Of Coleoptile Growth
2. Inhibition Of Seed Germination
3. Inhibition Of Gibberellin Action On α-Amylase Synthesis Germinating Cereal Grains
4. Promotion Of Stomatal closure if applied exogenously on leaves

Originally thought to be involved in regulating both abscission and bud dormancy, ABA actually has very less to do with either of these phenomena.

Two major areas of ABA action are:

1. **The induction of storage protein synthesis during seed development** and
2. **In regulating stomatal closure in times of water stress**.

During germination of cereal grains, ABA antagonizes the promotory effect of gibberellins on α-amylase synthesis.

The major physiological roles of ABA in plant physiology are summarized below.

1. ABA is an **efficient inhibitor of seed germination** and occurs in high concentrations in dormant seeds. ABA is **necessary for imposition and maintenance of seed dormancy**. In sprouting seeds, ABA content decreases and auxin, gibbrellin and CK concentrations peak up — an indication that germination is controlled by an equilibrium of auxin(s), gibberellin(s), and cytokinin(s) on one and ABA on the other hand. ***For details of this aspect of ABA physiology, please consult your notes on*** **SEED PHYSIOLOGY.**
2. ABA **reverses the effect of growth-stimulating hormones (auxin, gibberellins, cytokinin)** in several tissues. The synthesis of hydroxylases within germinating wheat seeds, for example, does not take place after the application of ABA. The importance of the ABA effect can be interpreted as an inhibitor that has the ability to **close down certain parts of the plant metabolism for a period of time, when stresses build up**. Since ABA is easily removed from tissues, is its effect reversible.
3. Substantial evidences establish that **increased ABA levels limit transpirational water loss by reducing or closing stomatal aperture**. During vegetative growth, roots of many angiosperms synthesize ABA and transport it into the shoots under conditions of water stress. ABA is an essential mediator in triggering plant responses to adverse environmental stimuli. This is known to occur in a number of crop plants which include rice, barley, soybean, tomato, cotton, and alfalfa. Leaf ABA content in wild plants increases

about 300 times with water stress. Upon rehydration, the ABA level ceases to increase and returned to pre-stressed levels.

Details of role of ABA in stomatal closure: It involves two stages: 1. ABA redistribution in the leaf cell from alkalinazation of xylary sap. 2. ABA signaling at the guard cells. When a plant endures water stress in drying soil, ABA is synthesized in the roots and translocated to the leaf through the transpiration stream. ABA is redistributed by a pH change in the apoplast of the leaf, and may also be synthesized by the guard cells themselves. These processes result in an increase of ABA levels around or inside guard cells. The increased concentration of ABA stimulates stomatal closure and reduces transpirational water loss from the leaf. Stomatal closure occurs when the accumulated K^+, Cl^-, and organic solutes are released from the guard cells into the external space. Because most of the K^+ salts accumulate in the vacuoles of guard cells in open stomata, these ions must pass through both the vacuolar and plasma membranes during stomatal closure.

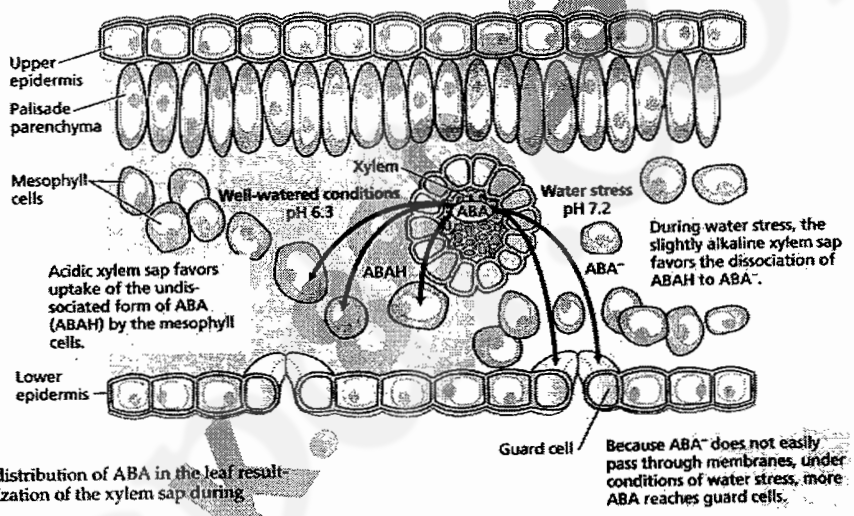


FIGURE 23.4 Redistribution of ABA in the leaf resulting from alkalinization of the xylem sap during water stress.

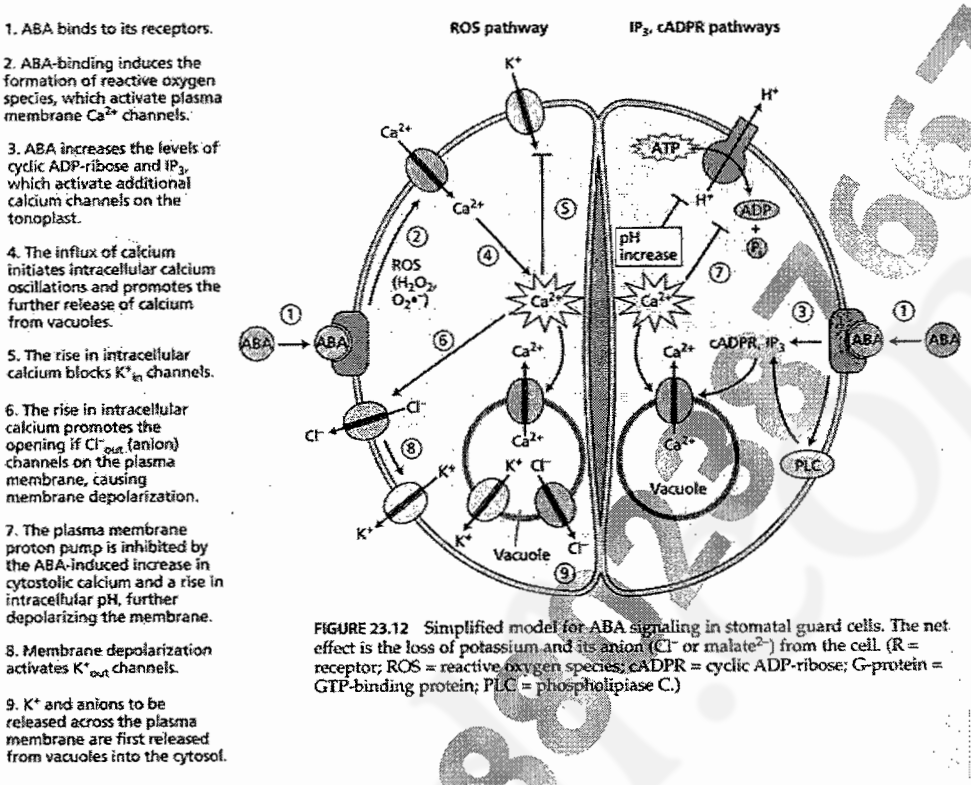


FIGURE 23.12 Simplified model for ABA signaling in stomatal guard cells. The net effect is the loss of potassium and its anion (Cl^- or malate^{2-}) from the cell. (R = receptor; ROS = reactive oxygen species; cADPR = cyclic ADP-ribose; G-protein = GTP-binding protein; PLC = phospholipiase C.)

4. Exogenous application of ABA was able to **increase plant adaptive response to various environmental stresses such as Water Stress, Chill Stress and salt Stress**. During extreme winters, ABA **imposes bud dormancy** in many trees to prevent frost injury.

5. ABA **prevents Vivipary** in most of the higher plants and the ABA mutants are frequently viviparous. In liverworts, a compound similar to ABA, named **lunularic acid** appears to play a dormancy related physiological role (in vegetative propagules called Gemma) similar to that of ABA in higher plants.

6. Although ethylene has been found to play a major role in the senescence of oat leaf segments, but ABA appears to be the initiating agent, whereas ethylene appears to exert its effects at a later stage.

Molecular Basis of ABA Action: In plants, heterotrimeric G protein signaling has earlier been linked to GA hormone responsiveness. However, in June 2004 issue of The Plant Cell, Pandey and Assmann provided strong evidence that *Arabidopsis* **G protein signaling also regulates ABA action**. In 2005, some ABA receptors have also been known to be present in the cytoplasm.

ABA initiates its effects on cells by binding to a receptor protein(s) — the activation of which causes a chain of events that results in • rapid changes in ion channels (of special significance in guard cells) and • slower changes in the pattern of gene transcription of **ABA response element (*ABRE*)** controlled genes. Many individual components of this chain of events have been identified (such as activation of PhospholipaseD or Activation of some transcription factors), but a complete picture has not been obtained yet. Since ABA is involved in responses to stress, there is a considerable overlap between the signaling processes induced by ABA and those initiated directly by stress.

Plant Hormones – VI: Brassinosteroids

Introduction to Brassinosteroids

Brassinosteroids (BRs) are steroid hormones ubiquitously found in plants but first purified from *Brassica*. The biologically active form this plant steroid is Brassinolide (BL).

To date, more than 50 BL analogs have been identified, and the group has been termed brassinosteroids (BRs).

BRs are remarkable in the diversity of their actions. BRs contribute to growth, vascular differentiation, reproductive development, and stress responses. They have potential applications in improving plant stress tolerances and increasing grain yields.

For their wide range of action, the BRs were suggested to be a sixth type of plant hormone, soon after their discovery. However, for years BRs were not considered true plant hormones. The turning point in BR research was the discovery of the *Arabidopsis* dwarf mutants *det2* and *cpd* in 1996 (Li et al., 1996; Szekeres et al., 1996). These BR-deficient mutants were found to revert to the wild-type phenotype following BR treatment.

Eventually, BRs were widely recognised as important plant hormones indispensable for growth and differentiation.

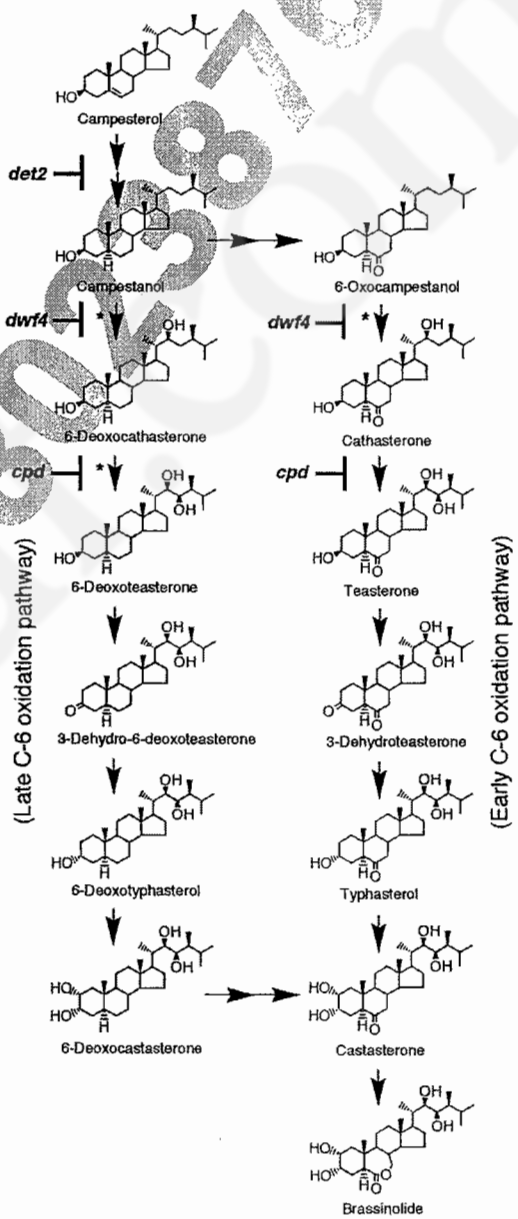


Figure 1: Brassinolide synthesis pathways

Occurrence

The Brassinosteroids are found at low levels in pollen, seeds, and young vegetative tissues throughout the plant kingdom.

Chemical nature

The BRs are polyhydroxylated derivatives of 5a-cholestane, structurally similar to cholesterol-derived animal steroid hormones and insect ecdysteroids.

Structurally, BRs are C27, C28, and C29 steroids with different substituents on the A- and B-rings and side chains. Brassinolide, the most biologically active BR, is a C28 steroid.

Synthesis of BRs

Evidences suggest that the plant steroid campesterol is the precursor of BRs.

In the 1990s BR biosynthesis and response mutants were identified, allowing the complete pathway of synthesis to be assembled. The roles of three genes in BR biosynthesis have been determined using dwarf mutants of *Arabidopsis*, *det2*, *cpd* and *dwf4*.

DET2 encodes a steroid 5α-reductase that hydrogenates the (24R)-24-methylcholest-4-en-3-one intermediate involved in the conversion of campesterol to campestanol (Fujioka et al., 1997).

CPD encodes a Cyt P450 enzyme, designated CYP90A1, that catalyzes C-23 hydroxylation (Szekeres et al., 1996), whereas DWF4 encodes a Cyt P450 enzyme, CYP90B1, that catalyzes C-22 hydroxylation (Choe et al., 1998).

Recently, brassinolide biosynthetic pathways have also been elucidated by feeding 2H-labeled intermediates to suspension cultures of *Catharanthus roseus*. The pathways of synthesis is shown in **Figure 1**.

Action of BRs

These hormones act synergistically, or at least additively, with several other hormones such as auxin and the gibberellins. However, they are far more potent than the other hormones — acting at far lower concentrations than the others.

BRs have been shown to be involved in numerous plant processes:

Promotion of cell expansion and cell elongation. In this action BR works with auxins.

It has an unclear role in cell division and cell wall regeneration.

Promotion of vascular differentiation; BR signal transduction has been studied during vascular differentiation.

Is necessary for pollen elongation for pollen tube formation.

Acceleration of senescence in dying tissue cultured cells; delayed senescence in BR mutants supports that this action may be biologically relevant.

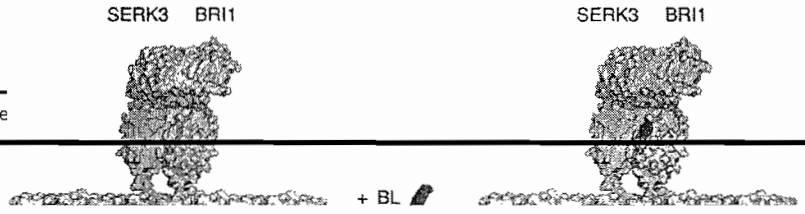
Can provide some protection to plants during chilling and drought stress.

Extract from the plant *Lychnis viscaria* contains a relatively high amount of Brassinosteroids. *Lychnis viscaria* increases the disease resistance of surrounding plants. In Germany, extract from the plant is allowed for use as a "plant strengthening substance."

Mutants unable to synthesize or unable to perceive BRs show dwarfed stature, impaired photomorphogenesis, and fertility defects (Clouse and Sasse, 1998).

BR signalling pathway

Unlike animal steroid signaling, which employs intracellular steroid receptors, in Arabidopsis (Arabidopsis thaliana), BR signaling is mainly mediated via the plasma membrane (PM)-located leucine-rich repeat (LRR) receptor-like kinase (RLK).



The leucine-rich repeat receptor-like kinase *BRASSINOSTEROID-INSENSITIVE1 (BRI1)* is the main receptor for brassinosteroids (BRs) in *Arabidopsis thaliana.* Binding of BRs to the ectodomain of plasma membrane (PM)-located BRI1 receptors initiates an intracellular signal transduction cascade.

Recently, it was shown that the initiation of BR signal transduction requires the interaction of BRI1 with its *SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE (SERK)* coreceptors. These co-receptors are also called *BRI1-ASSOCIATED KINASE1 [BAK1].*

Figure 2: Initial events in Brassinolide signaling

Current models of BR signaling assume that in the absence of ligands, BRI1 resides in a homodimeric configuration in the PM (Jaillais et al., 2011) and a double-lock mechanism prevents aberrant signaling activity.

Binding of BRs to the extracellular LRR domain of BRI1 homodimers results in conformational changes, which trigger BRI1 kinase activity. Subsequently, BRI1 transphosphorylate an inhibitor called BKI1, leading to the release of the inhibitor. The dissociation of BKI1 in turn enables the recruitment of BAK1(SERK3) into the BRI1 receptor complex (Christoph A. Bücherl et. al., 2013).

Via sequential transphosphorylation events within the BRI1-BAK1(SERK3) heterooligomers, BRI1 eventually gains full kinase activity and downstream BR signaling is initiated. It activates several signal intermediates, ultimately culminating in the transcriptional regulation of BR-responsive genes mediated by the *BRASSINAZOLE-RESISTENT1* (BZR1) and *bri1-EMS SUPPRESSOR1* (BES1; also known as BZR2) transcription factors.

Fruit Physiology

Introduction to fruits & their development

Botanically, the fruit is a mature ovary of the flower after fertilization. The wall of the ovary in the fruit is known as the *pericarp*, which becomes differentiated into three distinct layers: the outer *exocarp*, the middle *mesocarp*, and the inner *endocarp*.

Development of fruit after fertilization

Double fertilization is the trigger that evokes endosperm development and embryogenesis, transforming the ovule into a seed. As these changes take place in the ovule, the ovary increases in size and undergoes a variety of morphological, anatomical, and biochemical changes leading to the formation of a fruit with enclosed seeds.

Parthenocarpic fruit developmnt

Fruits such as banana (*Musa* sp.) and pineapple (*Ananas comosus*) may develop in the absence of fertilization of the egg, and such fruits will not harbor any seeds at all or will have only aborted seeds, whereas plants such as tomato have genetic lines that produce only seedless fruits. **Parthenocarpy** is the term used to describe the formation of fruits lacking seeds. It is now well known that the application of hormones such as auxins and gibberellins promotes parthenocarpic fruit development in apples (*Malus domestica*), grapes (*Vitis vinifera*), and tomatoes, among others.

Phases of fruit development

Tomato (*Solanum lycopersicon*) has become a popular model in which to study fleshy fruit development, because there are numerous mutants available and the plant is easily transformed. With tomato as a model, the development of fleshy fruits can be divided into five phases.

Phase I involves the development of the ovary in preparation for fertilization and seed development and ends with the decision to either abort further development or to proceed with further cell division and cell enlargement in the ovary walls. This decision to proceed with ovary development is generally referred to as *fruit set.*

Phase II is the initial phase of fruit development, *growth of the nascent fruit is primarily due to cell division* in the ovary walls. The cells thus become small and dense, with very small vacuoles.

Phase III: Now cell division effectively ceases and *further growth of the fruit is mostly by cell enlargement*. Once the fruit has reached its final size, it enters phase IV.

Phase IV is the period of ripening. In a fleshy fruit like tomato, ripening involves the development of color and flavour constituents (e.g., carotenoids, sugars, and acids) and a softening of the tissue that render the fruit attractive to animals. Tissue softening is primarily due to increased activity of enzymes such as polygalacturonase (PG). PG degrades the pectic substances that are found in the middle lamella and which are responsible for cell-to-cell adhesion.

Phase V: Now senescence sets in and the fruit begins the decay process.

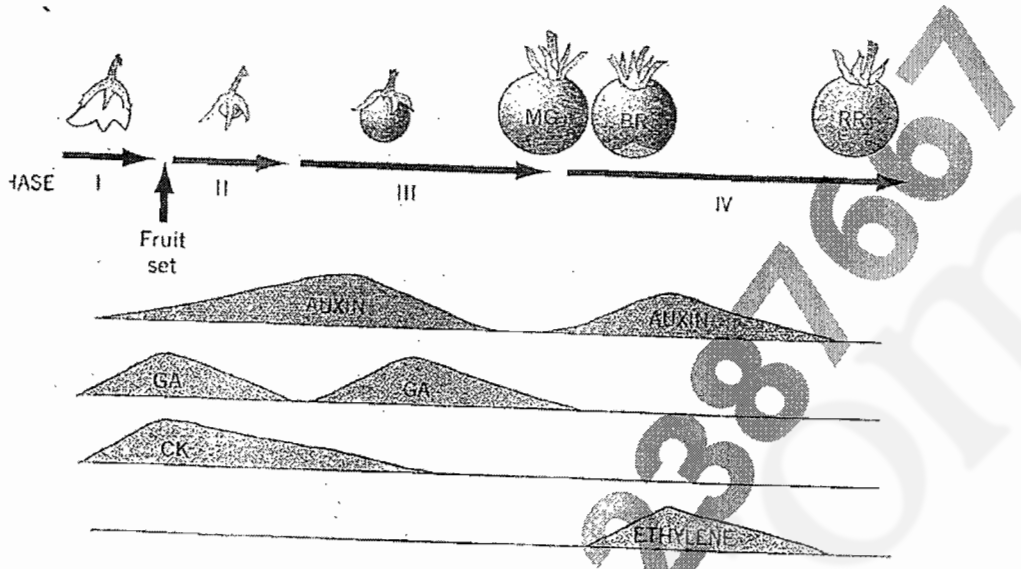


Figure 1: Hormonal changes during the first four phases of fruit development in a tomato. The graphs for auxin, gibberellin (GA), cytokinins (CK), and ethylene show the approximate point in development when hormone concentrations peak. Mature green (MG) is the fully grown unripe fruit. Breaker (BR) refers to the stage when the first signs of colour appear. Breaker marks the beginning of the ripening phase. The red ripe stage (RR) marks the end of ripening and the beginning of senescence.

Hormonal control of fruit development

All of the plant hormones except perhaps ABA are active at various stages during fruit development (Figure 1).

1. During seed development and first and second phases of fruit development, auxins, cytokinins, and gibberellins are all present and active.
2. Cytokinin level peaks during phase II, the period of most active cell division.
3. Auxin level peaks in early phase III, coinciding with the initiation of cell enlargement, and then declines as the fruit reaches mature size.
4. A second surge in auxin level occurs in the early stages of ripening, along with the appearance of significant levels of ethylene. Tomato is a climacteric fruit and the burst in respiration is related to the appearance of ethylene and the qualitative changes in the fruit that represent ripening.
5. The role of gibberellins is not well understood, but they are probably involved with cytokinins in initiating cell division and with auxin in maintaining cell enlargement.

Fruit ripening

Mature fruits undergo a late developmental transition involving coordinated changes in a number of catabolic and anabolic reactions that result in **ripening**. As a developmental process, the term "fruit ripening" is misleading in that it implies only the deteriorative aspects of the process resulting in spoilage and loss. Currently, *ripening is characterized as the final phase of fruit development reflecting harmonious changes in a number of biochemical pathways*. Among the changes are – (i) respiration, (ii) ethylene output, (iii) carotenoid synthesis, (iv) chlorophyll degradation, (v) production of cell wall hydrolases, and the (vi) softening process. Some fruits

not only evolve ethylene during ripening, but the ripening process itself is unwittingly accelerated by exogenous ethylene.

Climacteric and non-climacteric fruits

A rise in respiration, known as the **respiratory climacteric**, is one of a number of metabolic changes that occurs with the onset of ripening of fruits such as apple, avocado, banana, and tomato. Such fruits – **climacteric fruits** – contrast sharply with citrus (*Citrus* sp.) and lemon (*Citrus limon*), which do not show a respiratory climacteric and are hence classified as **non-climacteric fruits**.

Climacteric	Nonclimacteric
Apple	Bell pepper
Avocado	Cherry
Banana	Citrus
Cantaloupe	Grape
Cherimoya	Pineapple
Fig	Snap bean
Mango	Strawberry
Olive	Watermelon
Peach	
Pear	
Persimmon	
Plum	
Tomato	

Climacteric fruits show a large increase in ethylene production at the onset of ripening. After the ripening process is under way, ethylene output reaches a peak and continues at a relatively high level throughout the period of ripening. In addition, exogenous ethylene causes the rate of ethylene production to increase and advance the onset of respiratory climacteric in the fruits. Ethylene has long been known as a ripening hormore for climacteric fruits because, besides its effects on respiration, the gaseous hormone stimulates other ripening processes such as changes in color and softening of fruits.

Non-climacteric fruits do not produce ethylene as a part of their ripening program; although exogenous ethylene causes increases in respiratory activity in non-climateric fruits, it does not promote the natural ripening of such fruits.

Biochemistry of ripening

The unmistakable **signs of ripening** are the changes in – (a) color, (b) texture, (c) degree of acidity, and (d) aroma of the fruit that make it palatable. The following biochemical changes are important in this regard.

1. Major factors in the color change of fruits are the transition of chloroplasts into chromoplasts rich in yellow or red carotenoid pigments and increase in the water-soluble anthocyanins.
2. In ripening fruits, there is a decrease in acidity is associated with an increase in sugar content.
3. The soft texture of ripening fruits is due to increased activity of enzymes such as polygalacturonase (PG). PG degrades the pectic substances that are found in the middle lamella and which are responsible for cell-to-cell adhesion. Another important enzyme is Pectin Methyl Esterase (PME), which is a remarkably versatile enzyme that causes pectin deformation of the middle lamella of plant cell walls. The activity of the enzyme has been reported to increase during the development of fruits such as avocado, apple, banana and papaya.

4. Production of volatile compounds by the fruit during ripening is an important factor in the flavor of fruits. A diverse range of volatile compounds in varying quantities is released by each type of fruit. Organic acids, alcohols, esters, carbonyl compounds, hydrocarbons, and terpenoids are among the volatiles produced.

These specific changes suggest that, rather than being a deteriorative process, ripening involves a series of differentiation events. Earlier studies have also provided evidence that there is an increase in the protein content of ripening fruits. Fruits retain the capacity to synthesize proteins and RNA even as they begin to ripen, and inhibitors of protein and RNA synthesis prevent the progress of ripening of fruits.

Molecular biology of ripening

The characterization of genes involved in the chloroplast-chromoplast transition and the cell wall softening process during fruit ripening has been done in recent years. The main findings are as follows.

- Gene expression during ripening of fruits is marked by a change in the population of specific translatable mRNAs.
- During the ripening of tomato fruit, transcript levels of various genes coding for proteins of photosystem I, Photosystem II and the stroma decrease to undetectable levels.
- The accumulation of the carotenoids lycopene and β-carotene accounts for the red pigmentation of ripe tomatoes. Although many enzymes are involved in the multistep isoprenoid pathway of carotenoid biosynthesis, the PSY gene from tomato fruit, which encodes phytoene synthase, the first enzyme in the carotenoid pathway, is the main gene activated during ripening.
- The genes concerned with cell wall metabolism during fruit ripening are genes for to cellulase, Pectin Methyl Esterase (PME), and polygalacturonase.
- Continuing research has resulted in the cloning of additional ripening genes from tomato fruits. Expression of one of these genes, designated as E8, is activated during normal fruit ripening as well as when unripe fruits are exposed to ethylene. It is believed that the action of the E8 gene encodes a transcription factor.
- Closely related to the E8 gene is the E4 gene, whose transcripts are also abundant in ripening tomato fruits.
- Genes that regulate the ripening of other fruits than tomato have hardly been identified. Limited screening of cDNA libraries has led to the isolation of genes for cytochrome P-450 from avocado, pectin lyases, endochitinase, β-1,3-glucanase, thaumatinlike protein, ascorbate peroxidase, metallothionein, ethylene-forming enzyme ACC oxidase, and a senescence-related protein from banana, pectate lyases and HSP from strawberry, chitinase and thaumatin-like protein from grape, and endo-β-1, 4-glucanases from *Prunus persica* (Peach). The transcripts of these genes begin to accumulate at low levels in the respective unripe fruits and become moderately abundant in the ripe fruits.

Hormonal control of ripening

There is much experimental support for the view that **ethylene is involved in the ripening of climacteric fruits**. It is established that there is an important role of ethylene in the regulation of expression of E8 and E4 genes during fruit ripening.

The E8 and E4 transcription factors then control other genes related to ripening.

Recently, an analysis of mRNA from tomato fruits from wild-type and transgenic tomato plants genetically engineered to lack ethylene has revealed that gene expression during ripening is regulated by at least two independent pathways:

1. *An ethylene-dependent pathway* includes genes involved in lycopene and aroma biosynthesis, respiratory metabolism, and ACC synthase.
2. *A developmental, ethylene-independent pathway* includes genes encoding ACC oxidase and chlorophyllase.

Thus, not all of the processes associated with ripening intomato are ethylene dependent.

Ethylene works on target cells using a **two component signaling pathway**, as shown below. Significantly, the receptors are located in the ER membrane and they get negatively regulated by Ethylene. The main receptors for Ethylene are ***ETR1, ETR2, ERS 1, ERS 2*** and ***EIN4***. Please see mechanism of Ethylene Action for details.

Manipulations of fruit ripening

For a number of commercial considerations fruit ripening might be required to be manipulated. These considerations are:

1. Introducing the fruits early in the market for better returns.
2. Achieving longer shelf life to minimize the waste during transportation and storage.
3. Supplying the fruit in the market even after the season has declined to command better returns.

Depending on the objective of the merchant, the process of fruit ripening might be required to be accelerated or delayed. The following manipulative methods are particularly used.

1. Several chemicals which release ethylene are used now-a- days for promotion of flowering in pine apple. Ethrel or Ethephon (2 chloroethyl phosphoric acid) is ethylene releasing chemical. It rapidly breaks down in water to produce ethylene. Other ethylene releasing chemicals are- BOH, ethylpopyl phosphate, monoethyl sulphate.
2. Exogenous application of Auxins and ACC for triggering *in-vivo* ethylene biosynthesis.
3. Post harvest application of coal gas in rural storage areas to accelerate the ripening of mangoes.
4. Low temperature, low O_2 and high CO_2 levels are maintained in cold storages for inhibiting the synthesis and action of ethylene. Ripening process is considerably delayed in fruits like apples, avocado, peach etc.
5. In the field itself, inhibitors like methylcyclopropane can be applied which promotes the late ripening of fruits.
6. Post harvest, potassium permanganate can be applied to apples, bananas and pears to delay ripening.
7. By using rDNA technology, anti sense versions of ACC synthase and ACC oxidase genes have been introduced in some plants of commercial interest. This genetic transformation blocks ethylene synthesis and makes the fruit late ripening or never ripening. The common cultivated tomato (*Lycopersicon esculentum*) provides a major example of fruit ripening delay through genetic engineering. Identification of ripening-related cDNAs has enabled the modification of specific aspects of ripening by manipulating gene expression in transgenic lines of tomatoes. By utilizing antisense RNA to modify expression of ripening genes, the scientists have inhibited the production of the cell wall digesting enzymes polygalacturonase and pectinesterase and created transgenic plants with late ripening fruits. The *FlavrSavr* tomato was the first commercially grown genetically engineered food to

be granted a license for human consumption. It has delayed ripening and more resistant to rotting by adding an antisense gene which interferes with the production of the enzyme polygalacturonase.

8. Genetic lines of parthenocarpic plants are also helpful in delaying ripening. Since they do not have the seeds which are the principal ethylene sources.
9. Two potent ethylene inhibitors AVG. and trans-cyclooctane are not used. AVG is poisonous to humans and strongly mutagenic and trans-cyclooctane has very offensive smell.

Seed Physiology

Introduction to seeds

Seed is the term applied to the fertilized ovule of a seed plant before germination, which consists of an embryo and stored food material to support early development after germination. Surrounding the seed is a hard, tough *seed coat*, derived from the integument of the ovule and known as the *testa*. In angiosperms a second seed coat occurs within the testa. This second coat is thin and membranous and is known as the *tegmen*.

Seeds are small in size and make negligible demands upon their environment. Thus, they are eminently suited to perform a wide variety of functions: multiplication, perennation (surviving seasons of stress such as winter), dormancy (a state of arrested development), and dispersal. Pollination and the seed habit are considered the most important factors responsible for the overwhelming evolutionary success of the flowering plants, which have more than 300,000 species.

The advantage of dispersal by means of seeds rather than the spores lies mainly in two factors: the stored reserve of nutrient material that gives the new generation an excellent growing start and the seed's multicellular structure, which provides ample opportunity for the development of adaptations for dispersal, such as plumes for wind dispersal, barbs, and others.

Seed Development

Developmentally, the seeds are nothing but fertilized ovules containing the embryo, reserve food materials and being surrounded by a protective coat.

Brief outline of angiosperm fertilisation leading to the formation of seed

During the process of fertilization the pollen tube enters the ovule through a small opening known as the *micropyle*. One of the two sperm nuclei in the pollen tube unites with the egg cell in the ovule to form a zygote, which develops into the embryo. In angiosperms, the other sperm nucleus unites with two polar nuclei present in the embryo sac to form an endosperm nucleus, which later produces the nutritive endosperm tissue surrounding the embryo in the seed. In gymnosperms, the endosperm is formed from the tissue of the embryo sac itself.

The *nucellus*, or megasporangium, is the tissue composing the main part of the ovule; it is partially digested during the development of the embryo and endosperm tissue. As the embryo and endosperm are maturing, the ovule is also said to be maturing into a seed. One of the important transitions occurring during the late stages of embryogenesis is that the ovular integuments begin to mature into seed coats.

Surrounding a mature seed is a hard, tough seed coat, derived from the integument of the ovule and known as the *testa*. In flowering plants a second seed coat occurs within the testa; this second coat is thin and membranous and is known as the *tegmen*. Some seeds, in addition, have projects from the seed coat which help in the absorption of water when the seed is about to germinate or may form an additional protective coating around the seed.

In almost every seed, the micropyle through which the pollen tube entered the ovule persists as a small opening in the seed coat. Close to the micropyle in flowering plants, a stalk, or *funiculus*, attaches the seed to the placenta on the inside of the fruit wall. When the seed is removed, a small scar, known as the *hilum*, marks the former attachment of the stalk.

Embryonic maturation within the seed

In most seed plants embryo development occurs prior to seed dispersal: the embryonic root, or *radicle*, usually grows toward the micropyle; the embryonic bud, called *plumule*, or *epicotyl*, is at the end of the embryo opposite to the radicle; the embryonic stem, or *hypocotyl*, connects the radicle with the seed leaves, or *cotyledons*.

In gymnosperms, several cotyledons are usually present; among angiosperms two great groups of plants exist, one group having one cotyledon in the seed and known as the *monocotyledons*, and the other with two cotyledons and known as *dicotyledons*. The cotyledons serve as centers of absorption and storage, drawing nutritive material from the endosperm. The cotyledons of many plants, such as the sunflower, function as primary photosynthetic organs after germination and before the development of foliage leaves from the plumule.

In a few plants, such as the orchids, the embryo is a small, undifferentiated mass of cells till the time of seed release from the parent plant. It is during the period between separation from the parent plant and eventual germination, the undifferentiated cells develop into an embryonic root, bud, stalk, and leaf.

Seed dormancy

Seed germination is the resumption of growth of the embryo of the mature seed; it depends on the same environmental conditions as vegetative growth does. Water and oxygen must be available, the temperature must be suitable, and there must be no inhibitory substances present. However, in many cases a viable (living) seed will not germinate even though all the necessary environmental conditions for growth are satisfied. This phenomenon is termed **seed dormancy.** Seed dormancy introduces a temporal delay in the germination process that provides additional time for seed dispersal over greater geographical distances. It also maximizes seedling survival by preventing germination under unfavorable conditions.

As mentioned above, dormancy is an adaptive feature found in most of the seed plants because it offers several advantages including:

1. Avoidance of germination under unfavourable conditions
2. A time lag to ensure germination in appropriate conditions
3. A time lag for long distance dispersal
4. By light dependency for germination in some species, dormancy also ensures that a seed is germinating at a proper location where solar radiation is optimally available. This feature is especially helpful in densely vegetated habitats.

Dormancy period is genetically controlled in plant species, although not many genes controlling this feature have been identified so far.

Seeds also fail to germinate, when the surrounding conditions are not favourable. However, this failure to germinate is not regarded as dormancy because if favourable conditions are created the seed will readily germinate. Therefore, seeds otherwise capable of germination but not actually in a germinative state because of some lacking environmental factor are known by a different name, i.e. *Quiescent Seeds.*

Primary and Secondary Seed Dormancy

Different types of seed dormancy also can be distinguished on the basis of the timing of dormancy onset rather than the cause of the dormancy.

1. Seeds that are released from the plant in a dormant state are said to exhibit primary dormancy.
2. Seeds that are released from the plant in a nondormant state but which become dormant if the conditions for germination are unfavorable exhibit secondary dormancy. For example, seeds of *Avena sativa* (oat) can become dormant in the presence of temperatures higher than the maximum for germination, whereas seeds of *Phacelia dubia* (small-flower scorpionweed) become dormant at temperatures below the minimum for germination. The mechanisms of secondary dormancy are poorly understood.

Types of Seed Dormancy based on causal Factors

Seed dormancy is defined as a state in which seeds are prevented from germinating even under environmental conditions normally favorable for germination. These conditions are a complex combination of water, light, temperature, gasses, mechanical restrictions, seed coats, and hormone structures.

During seed maturation, a mature embryo enters a quiescent phase in response to desiccation and then it slowly passes into a dormant state.

Two types of seed dormancy have been recognized, *coat-imposed dormancy* and *embryo dormancy.*

Coat imposed dormancy

Coat-imposed dormancy is dormancy imposed on the embryo by the seed coat and other enclosing tissues, such as endosperm, pericarp, or extrafloral organs. The embryos of such seeds will germinate readily in the presence of water and oxygen once the seed coat and other surrounding tissues are either removed or damaged.

There are five basic mechanisms of coat-imposed dormancy:

1. *Prevention of water uptake.* Prevention of water uptake by the seed coat is a common cause of seed dormancy in families found in arid and semiarid regions, especially among legumes, such as clover (*Trifolium* spp.) and alfalfa (*Medicago* spp.). Waxy cuticles, suberized layers, and lignified sclereids all combine to restrict the penetration of water into the seed.
2. *Mechanical constraint.* The first visible sign of germination is typically the radicle breaking through the seed coat. In some cases, however, the seed coat may be too rigid for the radicle to penetrate. Nuts with hard, lignified shells are examples of dormancy caused by mechanical constraint. Such shells must be broken by biotic or environmental forces for the seed to germinate. Even nonlignified tissues, such as the endosperm of lettuce seeds, can suppress expansion of the embryo. For the seeds to

germinate, the endosperm cell walls must be weakened by the production of cell wall–degrading enzymes.

3. *Interference with gas exchange.* Some seed coats are considerably less permeable to oxygen than an equivalent thickness of water is— e.g., less by a factor of about 10^4 in seeds of cocklebur (*Xanthium pennsylvanicum*). This lowered permeability to oxygen suggests that the seed coat inhibits germination by limiting the oxygen supply to the embryo. In support of this idea, investigators can break the dormancy of such seeds either by making a small hole in the coat with a pin (without weakening the coat mechanically), or by treating the coat with concentrated oxygen.

4. *Retention of inhibitors.* The seed coat may prevent the escape of inhibitors from the seed. For example, growth inhibitors readily diffuse out of isolated *Xanthium* embryos, but not from intact seeds.

5. *Inhibitor production.* Seed coats and pericarps may contain relatively high concentrations of growth inhibitors that can suppress germination of the embryo. ABA is a common germination inhibitor present in these maternal tissues. In certain cases where repeated washing (leaching) removes dormancy, the effect is thought to be due to the loss of such inhibitory compounds.

   Natural inhibitors, which completely suppress germination (coumarin, parasorbic acid, ferulic acid, phenols, protoanemonin, transcinnamic acid, alkaloids, essential oils, and the hormone dormin) may be present in the pulp or juice of fruits or in various parts of the seed.

   Ecologically, such inhibitors are important in at least three ways. Their slow disappearance with time may spread germination out over several years (a protection against catastrophes). Furthermore, when leached out by rainwater, they often serve as agents inhibiting the germination of other competitive plants nearby.

In horticulture and agriculture, the coats of such seeds are deliberately damaged or weakened by man (*scarification*). In chemical scarification, seeds are dipped into strong sulfuric acid, organic solvents such as acetone or alcohol, or even boiling water. In mechanical scarification, they may be shaken with some abrasive material such as sand or be scratched with a knife.

Embryo dormancy

The second type of seed dormancy is **embryo dormancy**, which refers to a dormancy that is inherent in the embryo and is not due to any influence of the seed coat or other surrounding tissues.

Embryo dormancy is thought to be due to the presence of inhibitors, especially ABA, as well as the absence of growth promoters, such as GA (gibberellic acid). The loss of embryo dormancy is often associated with a sharp drop in the ratio of ABA to GA. In some cases, the cotyledons exert an inhibitory effect. Species in which the cotyledons exert an inhibitory effect include European hazel (*Corylus avellana*) and European ash (*Fraxinus excelsior*). A fascinating demonstration of the cotyledon's ability to inhibit growth is found in species (e.g., peach) in which the isolated dormant embryos germinate but grow extremely slowly to form a dwarf plant. If the cotyledons are removed at an early stage of development, however, the plant abruptly shifts to normal growth.

Embryo dormancy can be overcome by a number of chemicals (potassium nitrate, thiourea, and ethylene chlorhydrin) and plant hormones (gibberellins and kinetin). These agents have been used experimentally to break seed dormancy. Their mode of action is obscure, but it is known that in some instances thiourea,

gibberellin, and kinetin can substitute for light. In some cases, in which the cotyledons exert an inhibitory effect, embryo dormancy can be relieved by amputation of the cotyledons.

The mechanism of dormancy

Dormancy in seeds is controlled primarily by shifting ABA concentrations. During late stages, the relative concentration of GA is also a critical determinant of breaking of the dormancy. Basically, seed development has three major stages:

1. Embryogenesis, characterized by rapid cell division and cell differentiation. In this stage, the concentration of Auxins, CKs and GAs control the developmental physiology.

2. Cell divisions stop and storage compounds begin to accumulate. These storage compounds are mobilized from the vegetative parts of the plant using phloem translocation. The substances arrive in the seed in simpler forms but they polymerise in the seed. In this stage, ABA stimulates the storage of proteins in the seed. The role of ABA in complex carbohydrate accumulation is not experimentally established so far. [Harrow, Mollet et al. 2004].

3. In the final phase, the embryo prepares to proceed in dormancy and loses upto 90% of its total water contents. ABA action at this stage promotes the accumulation of a class of protein called Late Embryogenesis Abundant [LEA] proteins. These proteins provide desiccation tolerance to the embryo by preventing denaturation of the cellular macromolecules. After losing water, the seed enters a state of quiescence. Dormancy may or may not follow depending on the species.

Proteins Required for Desiccation Tolerance

LEA protein sequences are highly conserved among a wide variety of species. They are related to two other groups of proteins, the **RAB** (*r*esponsive to *A*BA) and **DHN** (*deh*ydr*in*) proteins. These protein classes accumulate in a variety of tissues experiencing cellular dehydration, e.g. maturing embryos and pollen, and vegetative tissues exposed to drought stress, including extreme desiccation of "resurrection plants" (*Craterostigma plantagineum*). The LEA, RAB, and DHN proteins are all water soluble, basic, rich in glycine and lysine, and low in hydrophobic residues. As a result they are extremely hydrophilic and stable to boiling. These features led Dure and colleagues (1989) to propose that they function specifically in the protection of membranes and proteins against desiccation damage, possibly by binding water tightly or providing hydrophilic interactions in the absence of free water, and by preventing crystallization and denaturation of cellular components through their ability to act as stabilizing "solvents," similar to the effects of sugars. One of these proteins confers increased osmotolerance in transgenic yeast.

Environmental Factors Control ling the Release from Dormancy

Various external factors release the seed from dormancy, and dormant seeds typically respond to more than one condition.

1. Many seeds lose their dormancy when their moisture content is reduced to a certain level by drying. This method of breaking seed dormancy is called **afterripening**, and is usually performed in a special drying oven. On the other hand, if the seed becomes too dry (5% water content or less), the effectiveness of afterripening is diminished.

2. Another factor that can release seeds from dormancy is low temperature, or **chilling**. Many seeds require a period of cold (0 to 10°C) while in a fully hydrated (imbibed) state in order to germinate. In temperate-zone species, this requirement is of obvious survival value, since such seeds will germinate not in the fall, but only in the following spring. Chilling seeds to break their dormancy is an old practice in horticulture and forestry and traditionally has been referred to as ***stratification***. This term is derived from the old agricultural practice of allowing seeds with a chilling requirement to overwinter outdoors in layered mounds of moist soil. Today the seeds are simply stored in a refrigerator.

3. The third external factor that plays an important role in breaking seed dormancy is **light**. Many seeds have a light requirement for germination, which may involve only a brief exposure, as in the case of lettuce, an intermittent treatment (e.g., succulents of the genus *Kalanchoe*), or even a specific photoperiod involving short or long days.

   Phytochrome is the main sensor for light-regulated seed germination. Interestingly, *all light-requiring seeds exhibit seed coat dormancy*, and removal of the outer tissues of the seed allows the embryo to germinate in the absence of light. Light enables the radicle to penetrate the seed coat. This penetration often involves some enzymatic weakening of the enclosing tissues.

Significance of Dormancy

Seed dormancy has at least three functions: (1) immediate germination must be prevented even when circumstances are optimal so as to avoid exposure of the seedling to an unfavourable period (*e.g.*, winter), which is sure to follow; (2) the unfavourable period has to be survived; and (3) the various dispersing agents must be given time to act. Accordingly, the wide variation in seed longevity is linked with various dispersal mechanisms employed, as well as with the climate and its seasonal changes.

ADDITIONAL INFORMATION

TEMPERATURE AS A FACTOR CONTROLLING DORMANCY

Many species require moisture and low temperatures; for example, in apples, when the cold requirement is insufficiently met, abnormal seedlings result. Others (cereals, dogwood) afterripen during dry storage. The seeds of certain legumes—for example, the seeds of the tree lupin, the coats of which are extremely hard and impermeable—possess a hilum with an ingenious valve mechanism that allows water loss in dry air but prevents re-uptake of moisture in humid air. Of great practical importance is stratification, a procedure aimed at promoting a more uniform and faster germination of cold-requiring, afterripening seeds. In this procedure, seeds are placed for one to six months, depending on the species, between layers of sand, sawdust, sphagnum, or peat and kept moist as well as reasonably cold (usually 0° to 10° C [32° to 50° F]). A remarkable "double dormancy" has thus been uncovered in lily-of-the-valley and false Solomon's seal. Here, two successive cold treatments separated by a warm period are needed for complete seedling development. The first cold treatment eliminates the dormancy of the root; the warm period permits its outgrowth; and the second cold period eliminates epicotyl or leaf dormancy. Thus, almost two years may be required to obtain the complete plant. The optimal temperature for germination, ranging from 1° C (34° F) for bitterroot to 42° C (108° F) for pigweed, may also shift slightly as a result of stratification.

Many dry seeds are remarkably resistant to extreme temperatures, some even to that of liquid air (□ 140° C or □ 220° F). Seeds of Scotch broom and some Medicago species can be boiled briefly without losing viability. Ecologically, such heat resistance is important in vegetation types periodically ravaged by fire, such as in the California chaparral, where the germination of Ceanothus seeds may even be stimulated. Also important ecologically is a germination requirement calling for a modest daily alternation between a higher and a lower temperature. Especially in the desert, extreme temperature fluctuations are an unavoidable feature of the surface, whereas with increasing depth these fluctuations are gradually damped out. A requirement for a modest fluctuation—e.g., from 20° C (68° F) at night to 30° C (86° F) in the daytime (as displayed by the grass Oryzopsis miliacea)—practically ensures germination at fair depths; and this is advantageous because a seed germinating in soil has to strike a balance between two conflicting demands, both depending on depth—on the one hand, germination in deeper layers is advantageous because a dependable moisture supply simply is not available near the surface; but, on the other hand, closeness to the surface is desirable because it allows the seedling to reach air and light rapidly and become self-supporting.

LIGHT AS A FACTOR CONTROLLING DORMANCY

Many seeds are insensitive to light, but in a number of species germination is stimulated or inhibited by exposure to continuous or short periods of illumination. So stimulated are many grasses, lettuce, fireweed, peppergrass (Lepidium), mullein, evening primrose, yellow dock, loosestrife, and Chinese lantern plant. Corn (maize), the smaller cereals, and many legumes, such as beans and clover, germinate as well in light as in darkness. Inhibition by light is found in chive, garlic, and several other species of the lily family, jimson weed, fennel flower (Nigella), Phacelia, Nemophila, and pigweed (Amaranthus). Sometimes, imbibed (wet) seeds that do not germinate at all in darkness may be fully promoted by only a few seconds or minutes of white light. The best studied case of this type, and one that is a milestone in plant physiology, concerns seeds of the Grand Rapids variety of lettuce, which is stimulated to germination by red light (wavelength about 660 nanometres) but inhibited by "far red" light (wavelength about 730 nanometres). Alternations of the two treatments to almost any extent indicate that the last treatment received is the decisive one in determining whether the seeds will germinate.

Ecological role of light

Laboratory experiments and field observations indicate that light is a main controller of seed dormancy in a wide array of species. The absence of light, for example, was found in one study to be responsible for the nongermination of seeds of 20 out of 23 weed species commonly found in arable soil. In regions of shifting sands, seeds of Russian thistle germinate only when the fruits are uncovered, often after a burial period of several years. Conversely, the seeds of Calligonum comosum and the melon Citrullus colocynthis, inhabiting coarse sandy soils in the Negev Desert, are strongly inhibited by light. The survival value of this response, which restricts germination to buried seeds, lies in the fact that at the surface fluctuating environmental conditions may rapidly create a very hostile micro-environment. The seeds of Artemisia monosperma have an absolute light requirement but respond to extremely low intensities, such as is transmitted by a two-millimetre- (0.08-inch-) thick sand filter. In seeds buried too deeply, germination is prevented. The responsiveness to light, however, increases with the duration of water imbibition. Even when full responsiveness to light has been reached, maximal germination occurs only after several light-exposures are given at intervals. Certain Juncus seeds have an absolute light requirement over a wide range of temperatures; consequently, they do not germinate under dense vegetation or in overly deep water. In combination with temperature, light (in the sense of day length) may also restrict germination to the most suitable time of year. In birch, for example, seeds that have not gone through a cold period after imbibing water remain dormant after release from the mother plant in the fall and will germinate only when the days begin to lengthen the next spring.

From ***Plant Physiology*** (Ed. 4, 2006) by Taiz and Zaiger

The Longevity of Seeds

Seed longevity is of practical importance because of ongoing efforts to preserve plant genetic resources for future agricultural crops by setting up seed gene banks. Claims of dormant seeds, viable for thousands of years have been made, but are highly controversial. Extreme examples of ancient seeds that have been reported to retain their viability include submerged lotus seeds found in a 3,000-year-old boat near Tokyo, barley seeds from the 3,000-year-old tomb of King Tutankhamen, and arctic lupine seeds associated with rodent burrows determined to be 14,000 years old. In most cases, however, a scientifically rigorous examination of the data has either disproved or raised serious doubts about the claimed antiquity of the seeds (Bewley and Black 1994).

Although the most sensational claims for seed longevity are almost certainly bogus, seeds of Canna compacta apparently can live for at least 600 years.

Almost nothing is known about the mechanisms that determine the longevity of seeds. If these mechanisms could be understood, humanity might someday be able to greatly increase the seed longevity of agriculturally important species and varieties, thereby enhancing our ability to preserve plant genetic resources for generations to come.

Seed Germination

The term *germination* is applied to the resumption of the growth of the seed embryo after the period of dormancy. Germination does not take place unless the seed has been transported to a favorable environment by one of the agencies of seed dispersal.

The primary conditions of a favorable environment are adequate water and oxygen and suitable temperature. Different species of plants germinate best in different temperatures; as a rule, extremely cold or extremely warm temperatures do not favor germination. Some seeds also require adequate exposure to light before germinating.

During germination, water diffuses through the seed coats into the embryo, which has been almost completely dry during the period of dormancy, causing a swelling of the seed; the swelling is often so great that the seed coat is ruptured. With the absorption of oxygen by the seed, energy is made available for growth.

The food stored in the endosperm or in the cotyledons is broken down by enzymes into simpler substances that are transported through the embryo to the various centers of growth. The radicle is the first portion of the embryo to break through the seed coat. It develops root hairs that absorb water and attach the embryo to particles of soil. The hypocotyl then lengthens, bringing the plumule and often the cotyledon or cotyledons above the surface of the soil. If the cotyledons are brought into light, they develop chlorophyll and carry on photosynthesis until the true foliage leaves develop from the plumule. In many plants, especially members of the grass family, the cotyledons never appear above the surface of the soil, and photosynthesis does not occur until true leaves develop; the plant meanwhile subsists on food stores in the seed. From the time of germination until the plant is completely independent of food stored in the seed, the plant is known as a seedling.

In order for germination to occur, certain requirements must be met which will vary from species to species. There are several factors affecting seed germination.

1. First and foremost, seed must be viable meaning it must be capable of germination.
2. Proper storage of seed also factors in on seed germination. Seed must be placed in proper environmental conditions - optimal moisture or hydration, proper temperature, and ample oxygen must all be considered.
3. Light also may or may not be needed for seed germination.
4. Finally, a seed's ability to overcome primary dormancy will cause seed germination.

If these conditions are met, germination can take place.

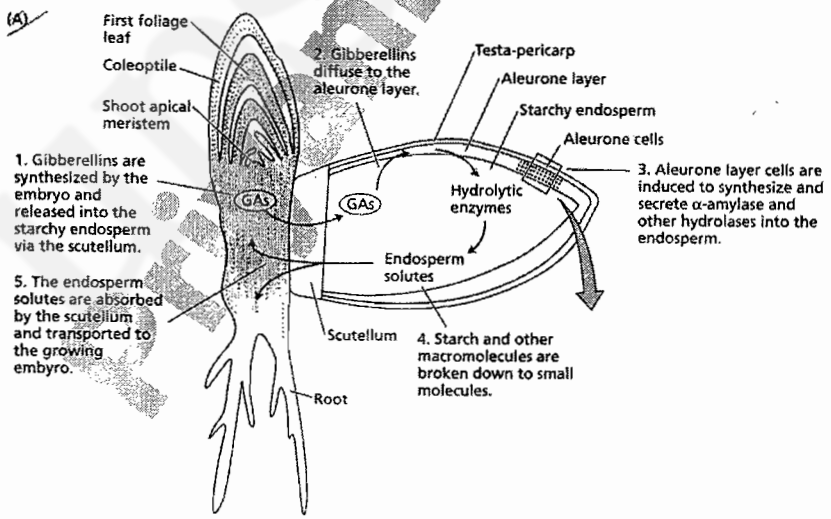


FIGURE 1: ACTIVATION FOR SEED GERMINATION

Seed germination follows three phases according to physiological processes.

Phase One – Activation

Imbibition of water is the first process that occurs during activation. Once imbibition of water has occurred, activation or the synthesis of enzymes is initiated. In most of the plant species, the activation phase

involves synthesis and release of Gibbrellic Acids (GAs) from the activated embryo. It is well studied in Barley seed germination (Fig. 1). The released GAs act on neighbouring cells and stimulate them to synthesize and release certain enzymes for example *alpha-amylase* in germinating barley seeds (Fig. 2 on Page 12). These enzymes function in the breaking down of storage material within the seed into simpler compounds such as sugars, which are utilized by the embryo for germination. Other enzymes activated during respiration start breaking down sugars for the production of energy that the developing seedling can use for growth and development. At the end of activation cell elongation and radicle emergence occur - the first visible (outward) sign that germination has commenced.

Phase Two - Digestion and Translocation

During digestion and translocation, enzymes that were synthesized or activated begin to break down storage material within the seed into simple compounds which are translocated to the embryo axis or plumule and root or radicle. The plumule will grow and develop as cells elongate and divide.

Phase Three - Seedling Growth

The germinating seed continues to undergo metabolic changes culminating into a seedling. Seedling growth can be of two types: epigeous germination or hypogeous germination. Both refer to the position of the cotyledons during germination. In epigeous germination (EPI - Latin meaning above or beyond), the cotyledons are pushed above the soil surface Beans and other legumes are examples. In hypogeous germination (HYPO - Latin meaning under), the cotyledons as well as most of the seed remains underground with only the shoot emerging from the soil surface.

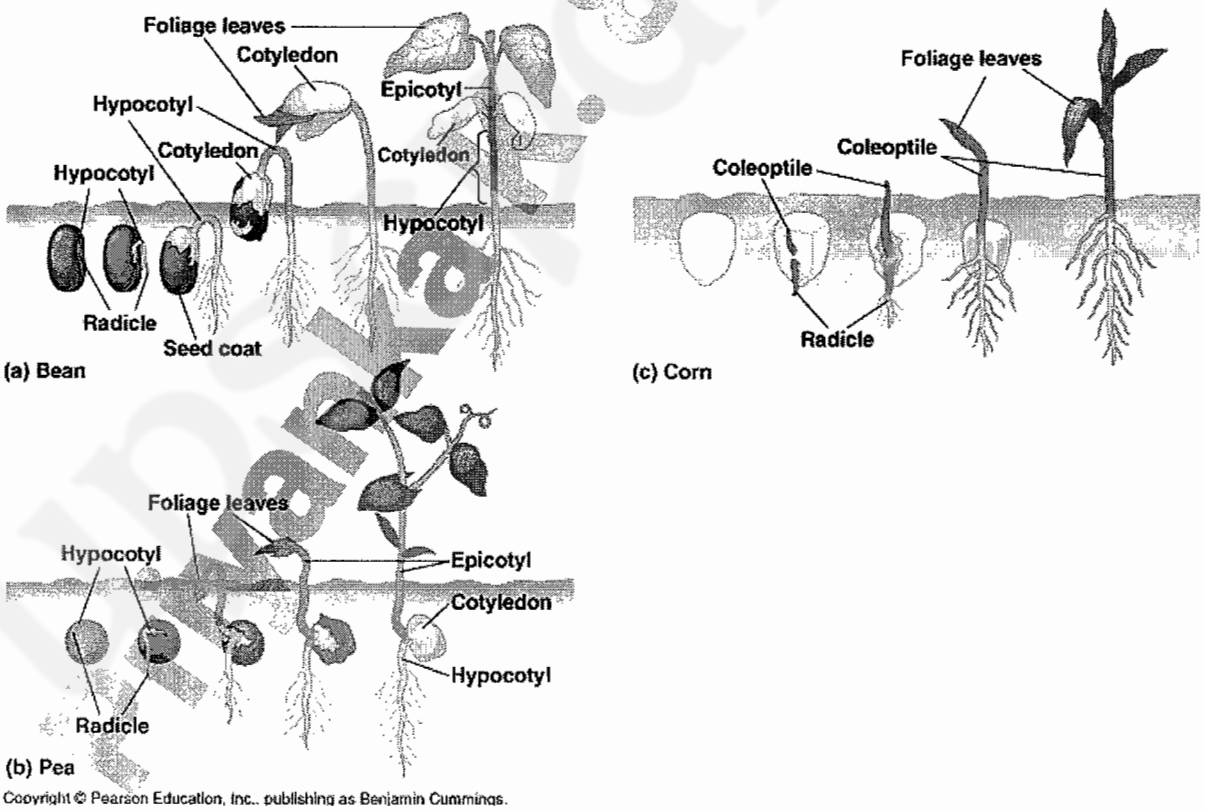


FIGURE 2: (A) EPIGEAL GERMINATION; (B) HYPOGEAL GERMINATION; (C) GRASS TYPE GERMINATION

Please refer to the Figure 4 on the next page for a step-wise description of cellular mechanism of GA action during seed germination – i.e. induction of synthesis and secretion of *Alpha-Amylase* enzyme in a germinating Barley seed.

1. GA_1 from the embryo first binds to a cell surface receptor.

2. The cell surface GA receptor complex interacts with a heterotrimeric G-protein, initiating two separate signal transduction chains.

3. A calcium-independent pathway, involving cGMP, results in the activation of a signaling intermediate.

4. The activated signaling intermediate binds to DELLA repressor proteins in the nucleus.

5. The DELLA repressors are degraded when bound to the GA signal.

6. The inactivation of the DELLA repressors allows the expression of the *MYB* gene, as well as other genes, to proceed through transcription, processing, and translation.

7. The newly synthesized MYB protein then enters the nucleus and binds to the promoter genes for α-amylase and other hydrolytic enzymes.

8. Transcription of α-amylase and other hydrolytic genes is activated.

9. α-Amylase and other hydrolases are synthesized on the rough ER.

10. Proteins are secreted via the Golgi.

11. The secretory pathway requires GA stimulation via a calcium–calmodulin-dependent signal transduction pathway.

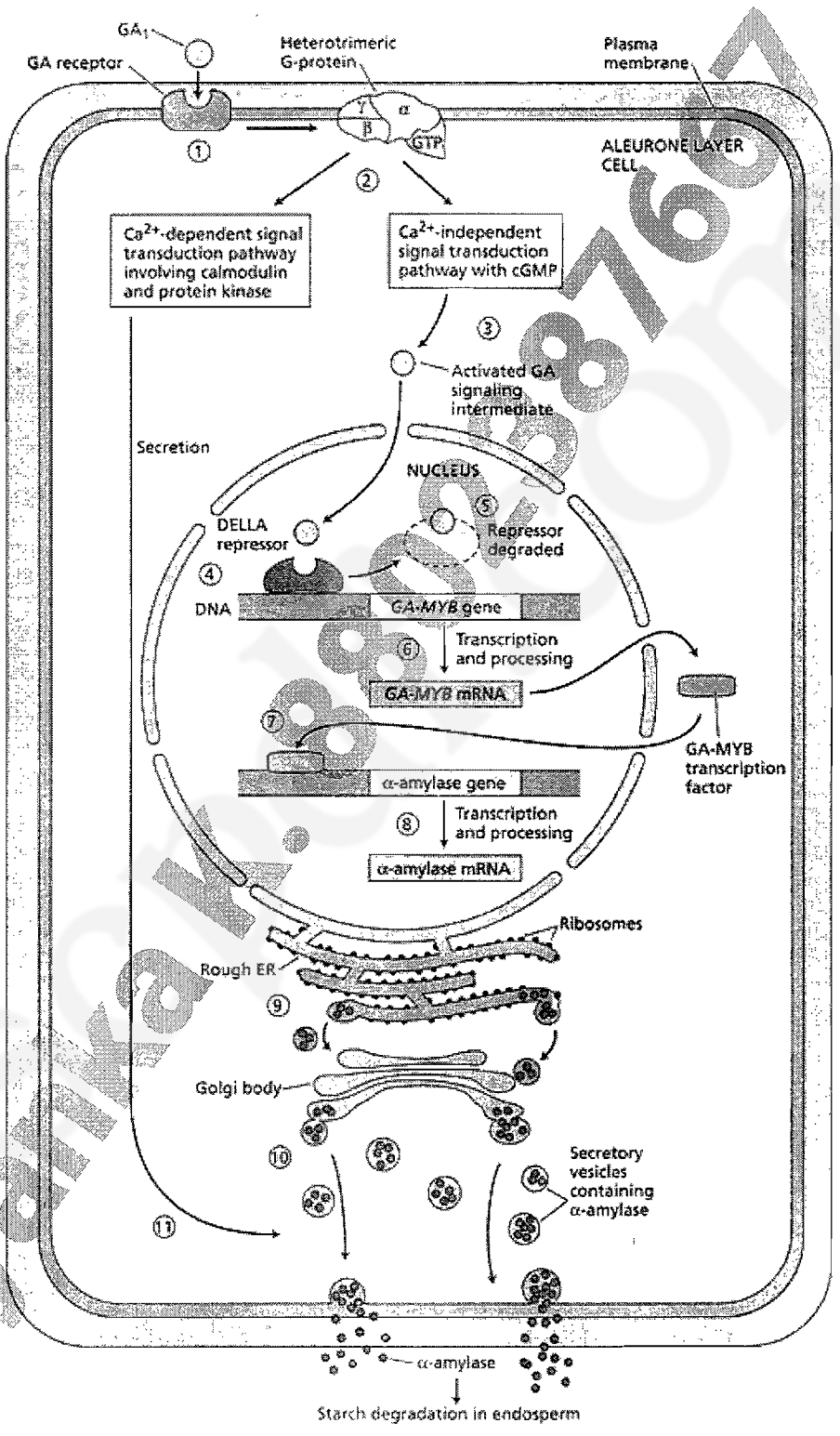


FIGURE 3: THE CELLULAR MECHANISM OF GA ACTION DURING SEED GERMINATION – I.E. INDUCTION OF SYNTHESIS AND SECRETION OF *ALPHA-AMYLASE* ENZYME IN A GERMINATING BARLEY SEED.

The important environmental factors controlling germination

Temperature

Plants may be classified into basically four groups in regard to the temperatures at which their seeds germinate. The first type is cool temperature tolerant plants which represent most plants that are native to temperate zones. Seed of cool temperature tolerant plants can germinate at temperatures as low as 4.5^0 C but prefer optimal temperature in the range of 25^0-30^0 C for germination to occur.

The second group of plants requires cool temperatures for seed germination. Seeds of this type originate from cool season plants, most of which are native to the Mediterranean or Mediterranean type climates. These climates usually have hot, dry summers and cool moist winters. In terms of seed germination, it is favorable for seed to germinate in winter under cool moist conditions rather than during the hot, dry summer. Thus plants of this group posses mechanisms that prevent their seed from germinating when soil temperatures exceed 25^0 C. The third group of plants require warm temperatures in order for seed germination to take place. Seed of this type generally will not germinate unless soil temperatures are 10-15^0 C and show visible signs of chilling injury if germination at lower temperatures occurs. Seedlings afflicted with chilling injury may be chlorotic, slow growing and accompanied by disease problems. Some examples of warm temperature requiring seeds include cotton and corn. Most of these plants are tropical in origin and adapted to regions where soil temperatures do not get very low.

The fourth group of plants require an alternating diurnal cycle of temperatures to initiate germinate. Basically, this involves alternating temperatures on the surface of the soil of warmth provided by sunlight during the day and cooling of soils at night through radiant heat loss.

Oxygen

Another factor affecting germination is concentration of oxygen available to the seed. Germinating seed are very metabolically active - respiration is occurring at high rates breaking down storage materials and sugars and converting them to other forms more useful to the seed. This all requires oxygen which is thus necessary for seed germination. Oxygen may be limiting in heavy soils such as clay or flooded conditions which in turn will affect seed germination.

Light

Another factor affecting germination in some types of seeds is light. Epiphytes are an examples of plants that require light in order for seed to germinate. Many epiphytic plants posses seeds that are light sensitive, meaning that if exposed to darkness for extended periods, viability is lost. In other words, light must be present in order for these seeds to germinate.

Light may also be a factor in physiological dormancy. Many plants having very small seeds require exposure to light to overcome physiological dormancy. Seed of this type must be on or near the soil surface in order to germinate. Lettuce is an example of a plant that generally needs exposure to light in order to germinate. Conifers as well need light requirements to satisfy seed germination.

Conversely, there are plants that need darkness in order for their seed to germinate. This is related to soil depth. Cacti and other desert plants are examples and are adapted so that germination only occurs in total darkness deep within the soil. The deeper the seed is in soil the closer it is in proximity to moisture which is a requirement for survival in desert environments.

Photoperiod or changes in daylength can also affect germination. Certain seeds, especially those of some woody, temperate species require particular daylengths before germination can occur.

Seed harvest

Embryo development progresses to the formation of seed produced within the fruit. Fruit maturity ultimately brings about dispersal of seeds to produce the next generation of plants.

In regard to propagation methods, seed harvest and extraction is dependent upon the method of seed dispersal and fruit type which can be divided into three categories:

Type I fruit includes mainly field crops with indehiscent seed. Indehiscent seed does not disperse but rather as the fruit ripens around the seed, the fruit stays in contact with the seed. Fruit of this type are often dry and will not split open to disperse seed even after maturity. Enclosed seed may be loose within the fruit.

Seed from type I category fruit are considered to be the easiest to harvest. Combines are commonly used to mechanically harvest these seed by removing the fruit from the plant and threshing to separate seed from fruit.

Type II fruit include plants such as vegetables, trees, and shrubs, that disperse their seeds through dehiscent fruit. Fruit from these plants will dry and crack open when mature, dispersing seed.

Type II fruit is generally harvested slightly immature before dehiscing, being much easier and less labor intensive than allowing the fruit to dehisce. Okra is an example.

In both Type I and Type II categories, the fruit is essentially dry at maturity.

In Type III fruit, seeds at maturity are surrounded by a fleshy fruit or pulp. Examples include: tomatoes, melons, citrus, pome fruits, and stone fruits.

Type III fruit requires the most labor and treatment to harvest and extract seed. With fleshy fruits, some methods of blending must be utilized in order to macerate the fruit. It is important to avoid seed injury in this process with rubber rather than steel blades used on mechanical blenders. After physically mashing the fruit, floatation is incorporated where water is added to the mix. Healthy, intact seeds being dense will sink to the bottom, while pulp will rise to the top of the mixture which can then be removed and the seeds collected. Floatation alone may be inadequate in removing fruit tissue surrounding the seed in which case an additional step of fermentation is added after floatation. The mixture of pulp and seed is allowed to sit for a couple of days to ferment or basically rot. The pulp is then removed by floatation and the seed is extracted. Plums are an example in which both fermentation and floatation are used to extract seed.

Cones

Cones do not really fit into the above categories and may be divided into dehiscent and delayed dehiscent.

With dehiscent cones, once the seeds inside the cone are mature, the cone opens dispersing the seed.

Delayed dehiscent cones are an adaptation to the coniferous plant allowing it to avoid environmental conditions such as forest fires that are adverse to seed germination. Certain pines in the western United States are adapted so that their cones only open during high temperatures caused by fire. Forest fires clear

underbrush allowing delayed dehiscent cone species to have increased germination and survival without competition from undergrowth.

On a commercial basis, cones are harvested immature and placed in dryers to trigger cones to open thereby releasing seed.

Seed life expectancy and storage

After harvest and extraction, seeds are often stored before planting. Viability or life expectancy of seeds are important considerations to make before storage. Two factors to consider concerning seed life expectancy are initial viability and long term viability. Viability usually does not improve but rather deteriorates with age. On the basis of viability seed can by divided into three categories: recalcitrant, orthodox, and long lived.

Recalcitrant

Recalcitrant seeds are short lived, usually remaining viable from a few days up to three years which may initially seem like a long period of time but in reality is considerably short. Examples include spring ripening seeds such as willow and elm, where the seed ripens in the spring, falls to the ground and germinates immediately. Seedlings then can develop over a long period through the summer and fall. Recalcitrant seeds tend to lose viability very rapidly and seed of this category are often harvested and planted immediately. Tropical plants also may posses recalcitrant seeds due in part to environmental conditions of high humidity and high temperatures are unfavorable conditions for maintaining seed viability and longevity. Other examples of plants possessing recalcitrant seed include aquatic plants, as well as nuts (e.g.:oaks, pecans, walnuts).

Orthodox

The largest group of plants fall into the category of having life expectancies ranging from two to three years up to fifteen years. Orthodox seed remain viable for longer periods because they are desiccation tolerant - seed may be dried down while maintaining viability. Some examples of orthodox seed include conifers, fruit trees, most vegetables and flowers, and grain crops.

Long Lived Seeds

Long lived seeds remain viable from fifteen to twenty years or more. Generally, long lived seeds have a mechanism in which their seed coats are impermeable to water (thick seed coats). Examples include certain types of weed seeds that can remain viable from fifty to one hundred years. An excellent example of a plant possessing long lived seed is the Indian Lotus. Seed of the Indian Lotus excavated and dated back to over 1,000 years have been successfully germinated.

Seed storage factors

Seed viability affects the method of storage. Two interrelated factors that contribute most to seed viability are moisture content and temperature.

Moisture Content

Moisture content in storage is dependent on the temperature at which the seed is stored and should be evaluated according to seed type - orthodox, recalcitrant, or long lived. For most orthodox seed, 4-6% moisture content is ideal for storage. Below 1-2% moisture content, damage begins to occur to seeds.

Conversely with 8-9% moisture content, insect activity increases, creating problems for stored seed. At 12-14% moisture content, fungal growth increases. Increasing moisture to18-20% moisture content causes seed metabolism to increase, increasing the rate of respiration. At 40-60% moisture content, the seed will initiate germination. In regard to specific storage conditions, generally, seed is kept at 20-25% relative humidity.

One reason recalcitrant seeds are short lived is that they are generally not desiccant tolerant. For example, maple seeds generally have a moisture content of about 58% at maturity. If the moisture content of maple seed drops below 30-34%, death occurs in the seed. In contrast, the moisture content in orthodox seed can drop down to 1-2% before damage occurs.

In regard to moisture, each 1% decrease in seed moisture (in the range of 5-14% for orthodox seeds) doubles life expectancy.

Temperature

Temperature also plays an important role in seed storage and viability. Generally, the lower the temperature, the higher the moisture content that can be tolerated by the seed in storage. As stated above, temperature is interrelated to moisture in maintaining seed viability. The general rule when dealing with temperature is that for every 5 o C decrease in temperature (in the range of 0-44° C) life expectancy in seed is doubled.

Most orthodox seed are usually stored under refrigerated conditions (non-freezing conditions). Occasionally, storage can be less than 0 o C which is beneficial for some seeds. It is critical to monitor for freezing injury of seed at low temperature storage to prevent freezing injury from occurring as a result of seed desiccation. Seed stored in this manner can become so dry that when the seed is exposed to moisture, water is imbibed too quickly. Seeds removed from cold storage should be allowed to equilibrate, slowly increasing moisture content and imbibition of water.

One further extreme for storing seeds in termed ultrafreezing or cryopreservation. Seeds cryopreserved are immersed in liquid nitrogen producing temperatures of minus 196 o C. Environmental control is very important and closely monitored with moisture content kept at 8-15% (depending on seed species). The rate of freezing is also very controlled over a period of hours. Removal of seed from cryopreservation is similarly controlled. With cryopreservation, seeds are stored in sealed containers and is mostly used for the storage of germplasm in plant breeding.

Types of storage

Seeds may be stored using varied methods and containers. The type of storage utilized is dependent on the use of the seed and budget. Seed storage methods include the use of open storage containers, sealed containers, conditioned storage, and moist, cool storage.

Open Storage

Open storage utilizes no temperature or moisture control. A simple shoebox on the shelf of a closet constitutes an open storage method. Open storage may be perfectly adequate for most orthodox seeds (e.g. vegetable and flower seeds). However, excess moisture, temperature extremes and insect and animal pests (rodents, rats) need to be carefully considered in open storage of seed.

Sealed Container

Sealed container storage of seed is simply placing seed into a container that can be closed and sealed. Sealed containers may or may not be moisture proof. Desiccants such as silica or cobalt chloride (which changes from pink to blue as moisture is absorbed) are often added to containers or mixed with the seed to absorb moisture. If a desiccant is mixed with the seed, the general rule of thumb is to add one part desiccant to ten parts seed. Moisture content in a sealed container should be kept in the range of 5% to 10%.

Conditioned Storage

Conditioned storage schemes for seed have moisture and temperature closely monitored. Generally, relative humidity is adjusted to 25-70%, depending on seed species. Atmospheric conditions may also be adjusted to reduce oxygen content in order to slow seed respiration and metabolic activity.

Moist, Cool Storage

Moist, cool storage is aimed primarily at recalcitrant seeds (seeds that are desiccation sensitive). Seed is often mixed with some type of moist media such as sand, potting media, or perlite - basically, any substrate that can hold some moisture around the seed. Temperatures are usually kept at 0-10o C (32 o -50 o F) and 80% to 90% relative humidity. Moist, cool storage is generally used for short term storage if seed sowing is delayed (e.g. bad weather).

Stress Physiology

Heat stress

Heat stress often is defined as where temperatures are hot enough for sufficient time that they cause damage to plant function or development.

Different plants are adapted to grow within different ranges of temperature optima. Accordingly, they have different ranges of temperature at which they will start experiencing heat stress.

Generally plants adapted to tropical conditions show heat stress symptoms at temperature above 45° C. The corresponding temperature levels for a temperate region plant is about 30° C or above and the same for a desert plant would be 55° C or above.

Manifestation of heat stress also depends on the plant type. For example, trees are more tolerant of heat in comparison to the herbaceous plants. Crop species and cultivars differ in their sensitivity to high temperatures. Cool-season annual species are more sensitive to hot weather than warm-season annuals. Table 1 contains examples of cool-season and warm-season annual crop species.

Table 1. Annual crop species adapted to cool and warm seasons (Hall 2001).

Cool-season annuals	Warm-season annuals
Barley, Brassicas, Canola, Fava Bean, Flax, Potato, Lentil, Lettuce, Mustard, Oat, Pea, Radish, Rye, Spinach, Turnip, Wheat	Common Bean, Cotton, Cowpea, Cucurbits , Finger Millet, Grain Amaranth, Maize, Mung Bean, Pearl Millet, Pepper, Pigeon Pea, Rice, Sesame, Sorghum, Soybean, Sunflower, Sweet Potato, Tobacco, Tomato

Development of heat stress

In field conditions, the heat stress develops due to:

1. Hot and clear sunny days
2. Slow wind conditions
3. Low levels of precipitation

Most often, water and temperature stress are interrelated. Shoots of most C3 and C4 plants with access to abundant water supply are maintained below 45°C by evaporative cooling; if water becomes limiting, evaporative cooling decreases and tissue temperatures increase.

Plants can be damaged in by either high day or high night temperatures and by either high air or high soil temperatures.

Effects of heat stress

Damages caused by heat stress

1. Both photosynthesis and respiration are inhibited at high temperatures, but as temperature increases, photosynthetic rates drop before respiratory rates. The temperature at which the amount of CO2 fixed

by photosynthesis, equals the amount of CO_2 released by respiration, in a given time interval is called the temperature compensation point.

At temperatures above the temperature compensation point, photosynthesis cannot replace the carbon used as a substrate for respiration. As a result, carbohydrate reserves decline, and fruits and vegetables lose sweetness. This imbalance between photosynthesis and respiration is one of the main reasons for the deleterious effects of high temperatures.

Enhanced respiration rates relative to photosynthesis at high temperatures are more detrimental in C3 plants than in C4 or CAM plants because the rates of both normal respiration and photorespiration are increased in C3 plants at higher temperatures.

2. High temperature reduces membrane stability. Excessive fluidity of membrane lipids at high temperatures is correlated with loss of physiological function. At high temperatures there is a decrease in the strength of hydrogen bonds and electrostatic interactions between polar groups of proteins within the aqueous phase of the membrane. High temperatures thus modify membrane composition and structure and can cause leakage of ions.
3. Membrane disruption also causes the inhibition of processes such as photosynthesis and respiration that depend on the activity of membrane-associated electron carriers and enzymes. O. Björkman and colleagues (1980) found that electron transport in photosystem II was more sensitive to high temperature.
4. Heat stress causes many cell proteins that function as enzymes or structural components to become unfolded or misfolded, thereby leading to loss of proper enzyme structure and activity. Such misfolded proteins often aggregate and precipitate, creating serious problems within the cell, including disturbance in the cellular metabolic processes.
5. High soil temperatures can reduce plant emergence. The maximum threshold temperatures for germination and emergence are higher for warm-season than for cool-season annuals. For example, the threshold maximum seed zone temperature for emergence of cowpea is about 37°C compared with 25 to 33°C for lettuce.
6. Extreme temperatures can cause premature death of plants. Among the cool-season annuals, pea is very sensitive to high day temperatures with death of the plant occurring when air temperatures exceed about 35°C for sufficient duration.
7. Reproductive development of many crop species is damaged by heat such that they produce no flowers or if they produce flowers they may set no fruit or seeds. The detrimental effects of heat stress on reproductive development that has been reported for cowpea, common bean, tomato, cotton, rice, wheat, maize and sorghum are well characterized.
8. Floral bud development also can be damaged by heat such that plants do not produce flowers. For cowpea, two weeks or more of consecutive or interrupted hot nights during the first month after germination caused complete suppression of floral bud development. In extreme cases the floral buds become necrotic and die. In field conditions, the damage occurs under long days but not under short days.
9. High temperatures can increase the rate of reproductive development, which shortens the time for photosynthesis to contribute to fruit or seed production.

Physiological adjustments towards heat stress

In general it has been observed that plants adapted to cool temperatures acclimate poorly to high temperatures.

1. In environments with intense solar radiation and high temperatures, plants avoid excessive heating of their leaves by decreasing their absorption of solar radiation. Heat resistance depends on certain adaptations:
    a. reflective leaf hairs
    b. leaf waxes
    c. leaf rolling
    d. vertical leaf orientation
    e. growth of small, highly dissected leaves to minimize the boundary layer thickness and thus maximize convective and conductive heat loss.

    Some desert shrubs—for example, white brittlebush (Encelia farinosa, family Compositae)—have dimorphic leaves to avoid excessive heating: Green, nearly hairless leaves found in the winter are replaced by white, pubescent leaves in the summer.

2. Many CAM, succulent higher plants, such as *Opuntia* and *Sempervivum*, are adapted to high temperatures and can tolerate tissue temperatures of 60 to 65°C under conditions of intense solar radiation in summer. Because CAM plants keep their stomata closed during the day, they cannot cool by transpiration. Instead, they dissipate the heat from incident solar radiation by re-emission of long-wave (infrared) radiation and loss of heat by conduction and convection.
3. In oleander (*Nerium oleander*), acclimation to high temperatures is associated with a greater degree of saturation of fatty acids in membrane lipids, which makes the membranes less fluid.
4. In response to sudden, 5 to 10°C rises in temperature, plants produce a unique set of proteins referred to as **heat shock proteins (HSPs)**. Most HSPs function to help cells withstand heat stress by acting as molecular chaperones. HSPs acting as molecular chaperones serve attain a proper folding of misfolded, aggregated proteins and to prevent misfolding of proteins. This facilitates proper cell functioning at elevated, stressful temperatures.
    The molecular masses of the HSPs range from 15 to 104 kDa (kilodaltons), and they can be grouped into five classes based on size. Different HSPs are localized to the nucleus, mitochondria, chloroplasts, endoplasmic reticulum, and cytosol. Members of the HSP60, HSP70, HSP90, and HSP100 groups act as molecular chaperones, involving ATP-dependent stabilization and folding of proteins, and the assembly of oligomeric proteins. Some HSPs assist in polypeptide transport across membranes into cellular compartments. HSP90s are associated with hormone receptors in animal cells and may be required for their activation, but there is no comparable information for plants.
    Low-molecular-weight (15–30 kDa) HSPs are more abundant in higher plants than in other organisms. Whereas plants contain five to six classes of low-molecular-weight HSPs, other eukaryotes show only one class (Buchanan et al. 2000). The different classes of 15–30 kDa molecular-weight HSPs (smHSPs) in plants are distributed in the cytosol, chloroplasts, ER and mitochondria. The function of these small HSPs is not understood.
    Cells that have been induced to synthesize HSPs show improved thermal tolerance and can tolerate exposure to temperatures that are otherwise lethal.
5. Periodic brief exposure to sublethal heat stresses often induces tolerance to otherwise lethal temperatures, a phenomenon referred to as **induced thermotolerance**. Conditions that induce thermal tolerance in plants closely match those that induce the accumulation of HSPs. This correlation plus several other evidences prove that HSPs play an essential role in acclimation to heat stress.

Salinity stress

Introduction to salinity stress

The two major environmental factors that currently reduce plant productivity are drought and salinity, and these stresses cause similar reactions in plants due to water stress.

Soil salinity, from agricultural stand point, is a condition when soil contains sufficient neutral soluble salts to adversely affect the growth of most crop plants. For purposes of definition, saline soils are those which have an electrical conductivity of the saturation soil extract of more than 4 dS/m at 25°C (Richards 1954).

In discussion of soil salinity, the scientists distinguish between high concentrations of Na^+, referred to as **sodicity**, and high concentrations of total salts, referred to as **salinity**. The two concepts are often related, but in some areas Ca^{2+}, Mg^{2+}, and SO_4^{2-}, as well as NaCl, can contribute substantially to salinity. The high Na^+ concentration of a sodic soil can not only injure plants directly but also degrade the soil structure, decreasing porosity and water permeability.

Soil salinity affects plant growth and development by way of osmotic stress, injurious effects of toxic Na^+ and Cl^- ions and to some extent Cl^- and SO_4^{2-} of Mg^{2+} and nutrient imbalance caused by excess of Na^+ and Cl^- ions.

Development of salinity stress

1. Under natural conditions, terrestrial higher plants encounter high concentrations of salts close to the seashore and in estuaries where seawater and freshwater mix or replace each other with the tides.
2. Far inland, natural salt seepage from geologic marine deposits can wash into adjoining areas, rendering them unusable for agriculture.
3. In inland deserts, evaporation and transpiration remove pure water (as vapor) from the soil, and this water loss concentrates solutes in the soil.
4. A much more extensive problem in agriculture is the accumulation of salts from irrigation water. When irrigation water contains a high concentration of solutes and when there is no opportunity to flush out accumulated salts to a drainage system, salts can quickly reach levels that are injurious to salt-sensitive species. It is estimated that about one-third of the irrigated land on Earth is affected by salt.

Effects of salinity stress

Plants can be divided into two broad groups on the basis of their response to high concentrations of salts.

1. **Halophytes** are native to saline soils and complete their life cycles in that environment. Some species that are highly tolerant of salt, such as *Suaeda maritima* (a salt marsh plant) and *Atriplex nummularia* (a saltbush), show growth stimulation at Cl^- concentrations many times greater than the lethal level for sensitive species.
2. **Glycophytes** or **nonhalophytes**, are not able to resist salts to the same degree as halophytes. Usually there is a threshold concentration of salt above which glycophytes begin to show signs of growth inhibition, leaf discoloration, and loss of dry weight.
   Among crops, maize, onion, citrus, pecan, lettuce, and bean are highly sensitive to salt; cotton and barley are moderately tolerant; and sugar beet and date palms are highly tolerant (Greenway and Munns 1980).

Plant physiologists currently identify four categories of plants, with respect to their sensitivity towards salinity. This categorization is based on studies on the growth of different species subjected to salinity for 1 to 6 months relative to that of unsalinized controls. (Greenway and Munns 1980.)

1. **Group IA (halophytes)** includes sea blite (*Suaeda maritima*) and salt bush (*Atriplex nummularia*). These species show growth stimulation with Cl^- levels below 400 nM.
2. **Group IB (halophytes)** includes Townsend's cordgrass (*Spartina townsendii*) and sugar beet (*Beta vulgaris*).These plants tolerate salt, but their growth is retarded.
3. **Group II (intermediate between halophytes and nonhalophytes)** includes salt-tolerant halophytic grasses that lack salt glands, such as *Festuca rubra* subsp. red fescue (littoralis) and *Puccinellia peisonis*, and nonhalophytes, such as cotton (*Gossypium* spp.) and barley (*Hordeum vulgare*). All are inhibited by high salt concentrations. Within this group, tomato (*Lycopersicon esculentum*) is intermediate, and common bean (*Phaseolus vulgaris*) and soybean (*Glycine max*) are sensitive.
4. The species in **Group III (very salt-sensitive nonhalophytes)** are severely inhibited or killed by low salt concentrations. Included are many fruit trees, such as citrus, avocado, and stone fruit.

Injuries due to salinity stress

1. Dissolved solutes in the rooting zone generate a low (more negative) osmotic potential that lowers the soil water potential. The general water balance of plants is thus affected because leaves need to develop an even lower water potential to maintain a gradient of water potential between the soil and the leaves. This effect of dissolved solutes is similar to that of a soil water deficit, and most plants respond to excessive levels of soil salinity in the same way as for water deficit. This conditions is known as **physiological draught**.
2. Specific ion toxicity effects also occur when injurious concentrations of ions—particularly Na^+, Cl^-, or $SO4^{2-}$— accumulate in cells. Under nonsaline conditions, the cytosol of higher-plant cells contains 100 to 200 mM K^+ and 1 to 10 mM Na^+, an ionic environment in which many enzymes function optimally. An abnormally high ratio of Na^+ to K^+ and high concentrations of total salts inactivate enzymes and inhibit protein synthesis. At a high concentration, Na^+ can displace Ca^{2+} from the plasma membrane of cotton root hairs, resulting in a change in plasma membrane permeability that can be detected as leakage of K^+ from the cells (Cramer et al. 1985).
3. Photosynthesis is inhibited when high concentrations of Na+ and/or Cl– accumulate in chloroplasts. Since photosynthetic electron transport appears relatively insensitive to salts, either carbon metabolism or photophosphorylation may be affected.

Adjustments towards salinity stress

1. Most plants can adjust osmotically when growing in saline soils. Such adjustment prevents loss of turgor while generating a lower water potential, but these plants often continue to grow more slowly after this adjustment.

   Two intracellular processes contribute to osmotic adjustment i.e. the decrease in Ψs, so that the plant can continue to take up water from the soil:

   a. the accumulation of ions in the vacuole
   b. the synthesis of compatible solutes in the cytosol.

   Compatible solutes include glycine betaine, proline, sorbitol, mannitol, pinitol, and sucrose. Specific plant families tend to use one or two of these compounds in preference to others. The amount of carbon used for the synthesis of these organic solutes can be rather large (about 10% of the plant weight). In natural vegetation this diversion of carbon to adjust water potential does not

affect survival, but in agricultural crops it can reduce growth and therefore total biomass and harvestable yields.

2. As mentioned earlier in the chapter, compatible solutes include glycine betaine, proline, sorbitol, mannitol, pinitol, and sucrose. Specific plant families tend to use one or two of these compounds in preference to others. The amount of carbon used for the synthesis of these organic solutes can be rather large (about 10% of the plant weight). In natural vegetation this diversion of carbon to adjust water potential does not affect survival, but in agricultural crops it can reduce growth and therefore total biomass and harvestable yields.
3. Casparian strip imposes a restriction to the movements of ions into the xylem. To bypass the Casparian strips, ions need to move from the apoplast to the symplastic pathway across cell membranes. This transition offers salt-resistant plants a mechanism to partially exclude harmful ions.
4. Plants minimize salt injury by excluding salt from meristems, particularly in the shoot, and from leaves that are actively expanding and photosynthesizing. Plants, such as salt cedar (*Tamarix sp.*) and salt bush (*Atriplex sp.*), have salt glands at the surface of the leaves. The ions are transported to these glands, where the salt crystallizes and is no longer harmful.
5. In general, halophytes have a greater capacity than glycophytes for ion accumulation in shoot cells.
6. In some plants, energy-dependent transport (efflux) of Na^+ from the cytosol of plant cells across the plasma membrane is mediated by the gene product of the SOS1 (*salt overly sensitive 1*) gene contributes to **salt exclusion**.
7. In tobacco (*Nicotiana tabacum*) cells adapted to grow under osmotic stress synthesize and accumulate a 26 kilodalton protein (**osmotin**) which can constitute as much as 12% of total cellular protein. In cells adapted to NaCl, osmotin occurs in two forms in the approximate ratio of 2:3.
    a. an aqueous soluble form (osmotin-I) and
    b. a detergent soluble form (osmotin II)

Both the osmotin types contribute to osmotic adjustment in glycophyte species.

Water stress

Introduction to water stress

Water stress may arise through either on excess of water or a water deficit. An example of excess water is flooding. Flooding stress is most commonly an oxygen stress, primarily due to reduced oxygen supply to the roots. Stress due to water deficit is far more common, so much so that the correct term water deficit stress is usually shortened to simply water stress. Since water stress in natural environments usually arises due to lack of rainfall, it is often referred to as Drought Stress.

Water stress can be defined as a condition when the soil water uptake is not enough to replenish the water lost by transpiration.

Injuries due to water stress

1. Water stress leads to **membrane damage**: Damage resulting from water stress is related to detrimental effects of desiccation on protoplasm. Removal of water from membrane disrupts the normal bilayer structure and introduce water filled channels lined with the phospholipids head groups. In other words, membranes become exceptionally porous when desiccated.
2. Photosynthesis is particularly sensitive to water stress: Photosynthesis can be affected by water stress in two ways. First, closure of the stomata normally cuts off access of the chloroplasts to the

atmospheric supply of carbon dioxide. Second, there are direct effects of low cellular water potential on the structural integrity of the photosynthetic machinery.

Water stress usually affects both leaf photosynthesis and stomatal conductance. As stomata close during early stages of water stress, water-use efficiency may increase (i.e., more CO_2 may be taken up per unit of water transpired) because stomatal closure inhibits transpiration more than it decreases intercellular CO_2 concentrations.

As stress becomes severe, however, the dehydration of mesophyll cells inhibits photosynthesis, mesophyll metabolism is impaired, and water-use efficiency usually decreases. Results from many studies have shown that the relative effect of water stress on stomatal conductance is significantly larger than that on photosynthesis.

3. Water stress indirectly decreases the amount of photosynthate exported from leaves. Because phloem transport depends on turgor, decreased water potential in the phloem during stress may inhibit the movement of assimilates.
4. As cytosol loses water, the ionic concentration increases. Cytosolic enzymes of plant cells can be severely inhibited by high concentrations of ions.
5. Enhanced root growth into moist soil zones during water stress requires allocation of assimilates to the growing root tips. During water deficit, assimilates are directed to the fruits and away from the roots. For this reason, the fruits grow much smaller during water stress.

Adjustments towards water stress

There are several adaptive types of plants with respect to water stress. The important behaviour shown by the plants include:

1. **Desiccation postponement**: the ability to maintain tissue hydration
2. **Desiccation tolerance**: the ability to function while dehydrated
3. **Drought tolerance**: survival at very low water potentials
4. **Drought escape**: comprises plants that complete their life cycles during the wet season, before the onset of drought.

The important mechanisms involved in Desiccation tor drought tolerance are as follows.

1. **Decreased leaf area** is an early adaptive response to water deficit. Cell expansion is a turgor-driven process and is extremely sensitive to water deficit. Because leaf expansion depends mostly on cell expansion, the principles that underlie the two processes are similar. Inhibition of cell expansion results in a slowing of leaf expansion early in the development of water deficits. The smaller leaf area transpires less water, effectively conserving a limited water supply in the soil over a longer period. Reduction in leaf area can thus be considered a first line of defense against drought.
2. In indeterminate plants, water stress limits not only leaf size, but also **leaf number**, because it decreases both the number and the growth rate of branches. Stem growth has been studied less than leaf expansion, but stem growth is probably affected by the same forces that limit leaf growth during stress.
3. If plants become water stressed after a substantial leaf area has developed, leaves will senesce and eventually fall off. Such a **leaf area adjustment** is an important long-term change that improves the plant's fitness in a water-limited environment. Many drought-deciduous, desert plants drop all their leaves during a drought and sprout new ones after a rain. This cycle can occur two or more times in a single season. Abscission during water stress results largely from enhanced synthesis of and responsiveness to the endogenous plant hormone ethylene.

4. Water deficit enhances **root extension** into deeper, moist soil. Mild water deficits also affect the development of the root system. Root-to-shoot biomass ratio appears to be governed by a functional balance between water uptake by the root and photosynthesis by the shoot. Simply stated, a shoot will grow until it is so large that water uptake by the roots becomes limiting to further growth; conversely, roots will grow until their demand for photosynthate from the shoot equals the supply. This functional balance is shifted if the water supply decreases.
5. **Stomatal closure**: Uptake and loss of water in guard cells changes their turgor and modulates stomatal opening and closing. Because guard cells are located in the leaf epidermis, they can lose turgor as a result of a direct loss of water by evaporation to the atmosphere. The decrease in turgor causes stomatal closure by **hydropassive closure**. This closing mechanism is likely to operate in air of low humidity, when direct water loss from the guard cells is too rapid to be balanced by water movement into the guard cells from adjacent epidermal cells.
   A second mechanism, called **hydroactive closure**, closes the stomata when the whole leaf or the roots are dehydrated and depends on metabolic processes in the guard cells. Abscisic acid (ABA) plays an important role in this process. (See the notes on *Abscisic acid (ABA)* for details).
6. **Osmotic adjustment** is a net increase in solute content per cell that is independent of the volume changes that result from loss of water. Compatible solutes include glycine betaine, proline, sorbitol, mannitol, pinitol, and sucrose. Specific plant families tend to use one or two of these compounds in preference to others.
   Several genes coding for enzymes associated with osmotic adjustment are turned on (up-regulated) by osmotic stress and/or salinity, and cold stress. These genes encode enzymes such as the following (Buchanan et al. 2000):
   - Δ'1-Pyrroline-5-carboxylate synthase, a key enzyme in the proline biosynthetic pathway
   - Betaine aldehyde dehydrogenase, an enzyme involved in glycine betaine accumulation
   - myo-Inositol 6-O-methyltransferase, a rate-limiting enzyme in the accumulation of the cyclic sugar alcohol called pinitol

   The amount of carbon used for the synthesis of these organic solutes can be rather large (about 10% of the plant weight). In natural vegetation this diversion of carbon to adjust water potential does not affect survival, but in agricultural crops it can reduce growth and therefore total biomass and harvestable yields.
7. A common developmental response to water stress is the production of a **thicker cuticle** that reduces water loss from the epidermis (cuticular transpiration). Although waxes are deposited in response to water deficit both on the surface and within the cuticle inner layer, the inner layer may be more important in controlling the rate of water loss in ways that are more complex than by just increasing the amount of wax present.
8. Under water stress conditions, to avoid excessive heat, in environments with intense solar radiation and high temperatures, plants respond by decreasing their absorption of solar radiation. **Heat resistance** depends on certain adaptations:
   a. reflective leaf hairs
   b. leaf waxes
   c. leaf rolling
   d. vertical leaf orientation
   e. growth of small, highly dissected leaves to minimize the boundary layer thickness and thus maximize convective and conductive heat loss.

9. Osmotic stress induces **Crassulacean Acid Metabolism** in some plants. A good example is *Mesembryanthemum crystallinum*, which shows the salt-induced osmotic stress caused shift from C3 metabolism to CAM. Crassulacean acid metabolism (CAM) is a plant adaptation in which stomata open at night and close during the day. The leaf-to-air vapor pressure difference that drives transpiration is much reduced at night, when both leaf and air are cool. As a result, the water-use efficiencies of CAM plants are among the highest measured. A CAM plant may gain 1 g of dry matter for only 125 g of water used—a ratio that is three to five times greater than the ratio for a typical C3 plant.
10. **Accumulation of LEA proteins** (named for *late embryogenesis abundant*), which are believed to play a role in cellular membrane protection. Although the function of LEA proteins is not well understood, they accumulate in vegetative tissues during episodes of osmotic stress. The proteins encoded by these genes are typically hydrophilic and strongly bind water. Their protective role might be associated with an ability to retain water and to prevent crystallization of important cellular proteins and other molecules during desiccation. They might also contribute to membrane stabilization.

Heavy metal stress

Based on their chemical and physical properties three different molecular mechanisms of heavy metal toxicity can be distinguished: (a) production of reactive oxygen species by autoxidation and Fenton reaction; this reaction is typical for transition metals such as iron or copper, (b) blocking of essential functional groups in biomolecules, this reaction has mainly been reported for non-redox-reactive heavy metals such as cadmium and mercury, (c) displacement of essential metal ions from biomolecules; the latter reaction occurs with different kinds of heavy metals.

Transition metals cause oxidative injury in plant tissue, but a literature survey did not provide evidence that this stress could be alleviated by increased levels of antioxidative systems. The reason may be that transition metals initiate hydroxyl radical production, which can not be controlled by antioxidants. Exposure of plants to non-redox reactive metals also resulted in oxidative stress as indicated by lipid peroxidation, H_2O_2 accumulation, and an oxidative burst. Cadmium and some other metals caused a transient depletion of GSH and an inhibition of antioxidative enzymes, especially of glutathione reductase. Assessment of antioxidative capacities by metabolic modelling suggested that the reported diminution of antioxidants was sufficient to cause H_2O_2 accumulation. The depletion of GSH is apparently a critical step in cadmium sensitivity since plants with improved capacities for GSH synthesis displayed higher Cd tolerance. Available data suggest that cadmium, when not detoxified rapidly enough, may trigger, via the disturbance of the redox control of the cell, a sequence of reactions leading to growth inhibition, stimulation of secondary metabolism, lignification, and finally cell death. This view is in contrast to the idea that cadmium results in unspecific necrosis. Plants in certain mycorrhizal associations are less sensitive to cadmium stress than non-mycorrhizal plants. Data about antioxidative systems in mycorrhizal fungi in pure culture and in symbiosis are scarce. The present results indicate that mycorrhization stimulated the phenolic defence system in the *Paxillus–Pinus* mycorrhizal symbiosis. Cadmium-induced changes in mycorrhizal roots were absent or smaller than those in non-mycorrhizal roots. These observations suggest that although changes in rhizospheric conditions were perceived by the root part of the symbiosis, the typical Cd-induced stress responses of phenolics were buffered. It is not known whether mycorrhization protected roots from Cd-induced injury by preventing access of cadmium to sensitive extra- or intracellular sites, or by excreted or intrinsic metal-chelators, or by other defence systems. It is possible that mycorrhizal fungi provide protection via GSH since higher concentrations of this thiol were found in pure cultures of the fungi than in bare roots. The development of

stress-tolerant plant-mycorrhizal associations may be a promising new strategy for phytoremediation and soil amelioration measures.

Abiotic stress (in particular heavy metals) often induce the synthesis and accumulation of the same defense-related secondary metabolites. Recently, a mechanism has been proposed that links reactive oxygen species (ROS) generation with lipid oxidation processes, ultimately resulting in the formation of similar, highly active signalling compounds. The generation of ROS is a common event in heavy metal stress.

Senescence

Senescence is ageing which is genetically controlled for its timing of incidence, progression of events, intrinsic control and ultimate outcome. Senescence can be defined as a process taking place in a time or age dependent manner which irreversibly leads to decreased physiological activity and eventual death.

Senescence is not necessarily degrading in its operation. There are several genes whose expression is stimulated in the senescent stages and many enzymes are also activated during senescent stages. Hence it is regarded as a controlled series of cellular and biochemical events whereby the organism or a particular part of it proceed towards physiological decline.

Senescence in Plants

Plants are unlike animals, with regards to senescence. The animals experience senescence at whole organism level while plants display it at four different levels-

1. Cellular senescence – It sets in under two conditions –
   (a) If the cell has attained its hay-flick limit, which is the genetically programmed maximum number of division of a cell can undergo, the cell must proceed towards senescence.
   (b) The cell may be responding to some development signal generated intrinsically. For e.g., the selective senescence of pericarp epidermal layer of the cells which is controlled by falling level of auxins and cytokinin & enhanced activity of ethylene.

2. Organ level senescence – Which is obvious in case of leaves which gradually turn yellow, become photosynthetically less active and behave as nutritional links. Fruit senescence and flower senescence are also well observed phenomenon. Organ senescence is almost always followed by organ abscission.

3. System senescence – In this type of behavior, it is only the shoot system which under goes senescence and eventually death. Well studied examples are herbaceous perennials which survive during harsh conditions by underground storage organs either through modified stems or a swollen root.

4. Whole organism senescence – Each plant has a genetically pre determined life span, in the last one third of which is mostly undergoes the senescence phase (Molisch, 1963). Available evidences support that seeds release ethylene which is the primary senescence hormone in plants.

Cellular Physiology of Senescence

In all organisms, senescence and programmed cellular death (PCD) are correlated. Senescence is always followed by PCD. It was first studied in animal in 1970's by John Kerr, Andrew Wylie et al. The animal cells committed to PCD are regarded as senescent and show the following characteristics –

1. Formation and pinching off as vesicles from the outer surface of the plasma membrane.
2. Vesicle emergence from nuclear envelope.
3. Chromatin condensation
4. Cleavage of DNA into characteristic 180 BP inter-nucleosomal fragments.
5. Formation of Apoptotic vesicles which are engulfed and degraded by neighboring cells.

In the plants, senesceing cells undergo internal reorganization but remain metabolically active. It is highly regulated process where new metabolic pathways are activated and others are turned off. In a summarized manner, the overall process triggered by hormonal, environmental and developmental factors proceeds in there phase –

Initiation phase

(a) Crossing of metabolic threshold
(b) Altered redox state of the cell (oxidation predominates)
(c) Initiation of signaling cascades.

Reorganization phase

(a) Activation of macro-molecular breakdown and salvage pathway (a process of preserving units for future biosynthetic activities in other tissues).
(b) Shift from autotrophic to heterotrophic metabolism.
(c) Organelle re-differentiation
(d) Detoxification

Terminal phase

(a) Antibiotic substance accumulates so that an opportunist pathogen does not enter the plant through the senescing tissue.
(b) Release of free radicles.
(c) Complete translocation of salvaged molecules.
(d) Elimination of remaining metabolites.
(e) Irreversible loss of cell integrity and viability (the last two cellular compartment to break down are nucleus and vacuoles).

Various workers have sub-divided the senescent physiology under two headings.

(i) Biochemical change
(ii) Gross- organelle level change

Biochemical changes

Studies on senescing tissue from various plants reveal 10 major biochemical changes in a senesceing cell –

(a) Break down of chlorophyll by the plastid located enzyme chlorophyllase.
(b) Selective break down of pigment system and LHC proteins which diminishes the photosynthetic rates in the tissue. After the above two changes the plant is called Gerontoplast.
(c) Change in phenyl propanyl metabolism and accumulation of tannins, flavinoids and lignin.
(d) Widespread protein breakdown for which two strategies are employed depending on the particular protein –
    (i) Removal of co-factor that increases the susceptibility for protease mediated degradation
    (ii) Ubiquitination after which a protein is broken down in proteasome complexes.

Small peptide fragments obtained from protein breakdown are acted upon by peptidases and free amino acids are generated.

(e) Export of organic nitrogen and organic sulphur out of the cell.
(f) Conversion of lipids into sugar Glyoxysomal enzymes, some of which are used as respiratory substrates and some can be exported.
(g) Amino acids are used as respiratory substrate at middle to late stages.
(h) Nucleic acids are broken are broken down to release inorganic phosphates and nitrogenous bases.
(i) Oxidative reactions in the cell predominate while reductive processes are minimal.
(j) Intra cellular signal transduction mostly operates in middle to late stages through reactive oxygen species (ROS)

Organelle level changes

These changes have been studied in detail in the cultured leaf mesophyll cells of Zinnia which were induced towards senescence and finally differentiated into tracheary elements. The important events include –

(a) Conversion of chloroplast into Gerontoplast which shrivel shortly after.
(b) Similar swelling and consequent shriveling of mitochondria.
(c) Some chloroplast and mitochondria may become enclosed within autophagic vacuoles and be broken down within the vacuoles.
(d) Emergence of thickening on the secondary wall followed by lignification of wall and PM and ultimate lysis of the PM.
(e) In the middle stages, peroxisomes are converted into glyoxysomes.
(f) ER forms small vesicles and ultimately disappears.
(g) GA forms tightly appressed cisternal systems and ultimately disappear.
(h) Nucleus disintegrates.
(i) The tonoplast membrane sulphare and the vacuole also disintegrates. The cytoplasmic components are translocated out or completely eliminated.

Hormonal control

On various plants hormonal activity has been examined during senescence. There are three principle conclusions –

(a) Ethylene is a primary senescence hormone by which many senescence associated genes are activated. Ethylene synthesis is self-sustainable and it triggers its own synthesis in neighbouring cells as well by stimulating a gene for ACC oxidase.
(b) In high concentration, Auxins stimulate ethylene synthesis hence it promotes senescence in mature tissues.
(c) CK is antagonist to senescence which is well established in Richmond Lang effect seen in leaves.

Senescence at gross level (organ level)

There are two well studied examples to serve as models for organ level senescence-

(I) Leaf senescence – Which is manifested as leaf abscission and the events preceding it. It has three stages of which the last two correspond to senescence in strict terms –

(a) Leaf maintenance phase where there is a gradient of auxin action between leaf blade and leaf petiole.

(b) Senescence induction where leaf blade stops acting as a source of auxins and petiole itself generates auxins. It has two outcomes –

- Increased CK sensitivity due to which rapid cell division occurs and abscission zone is created.
- Ethylene biosynthesis is stimulated due to which the newly generated cells have their walls and poorly developed protoplasm.

(c) Abscission stage – Where all the cellular process of senescence occurs and ultimately the leaf is detached from the parent plant through the abscission zone.

(II) Fruit senescence – Characterized by various events of fruit ripening i.e. primarily controlled by ethylene levels (for cellular details refer to *Fruit Physiology* notes).

Genetic control

Since senescence is an active and well regulated process, it involves the presence of twin mechanisms, i.e., expression of certain genes and repression of certain genes.

The genes whose expression is stimulated during senescence are called senescence associated genes (SAG) in *Arabidopsis thaliana* and senescence up regulated genes or SENU in tomato. The products of SAG or SENU classes of genesis include –

(i) Cysteine Protease
(ii) Ubiquitine carrier protein
(iii) Malate synthase
(iv) Isocitrate lyase
(v) Antibiotics etc.

There are several genes whose expression is repressed during senescence. They are comprehensively called SDG, senescence down regulated genes. The products of these includes –

(i) Pigment system proteins
(ii) Starch polymerizing enzymes
(iii) Nucleotide biosynthesis enzyme
(iv) Ribosomal proteins
(v) Chloroplast translocaters etc.

Correlation between Senescence and PCD

In the plants senescence and PCD are invariably correlated. The ultimate fate of each senesceing tissue is to undergo PCD. In some cases various internal factors or human manipulation can delay the incidence of senescence or the incidence of PCD. But PCD is unavoidable eventually. In higher plants, plant physiologists have studied the following nine instances where senescence and PCD are firmly correlated –

(i) PCD during megagametophyte formation
(ii) PCD in endosperm tissue
(iii) PCD of suspensor cells
(iv) PCD during trichome development
(v) PCD during tracheary element formation
(vi) PCD during leaf, fruit, floral organs senescence
(vii) PCD of root cap cells
(viii) PCD during aerenchyma formation
(ix) PCD during various lysogenous canal development and the formation of glandular tissues.

Senescence does not seem to be related to hypersensitive response, which is cellular death in case of a mechanical injury or pathogen inflicted damage. It is now established that the pathogen attack on a healthy plant cell by itself does not kill the cell but triggers a cellular mechanism by which the cell kills itself. Any young cell can also kill itself during hypersensitive response. It need not be senescent.

Significance of Senescence

1. Senescence is critical in normal development and reproduction of plants as shown in above 9 examples.
2. Organ senescence keeps the biomass appropriately regulated within a plant.
3. System or organ senescence can help the plant in avoiding unfavorable conditions. For eg., leaf abscission prevents frost injury in broad leaved temperate species. And shoot senescence avoids chill injury in herbaceous plants.
4. The plants get rid of nutritional sinks by senescence and PCD. For eg., in case of leaf abscission.
5. Fruit senescence is an important determinant in seed dispersal.
6. Whole plant senescence helps in population regulation within a community.
7. Time controlled whole plant senescence enables plant to develop under conditions when biotic component and other stresses are low.